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BOTRYTIS NECK ROTS OF ONIONS [1]

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INTRODUCTION

The serious nature of losses due to Botrytis infection of onion bulbs first came to the writer's attention in the late autumn of 1915. During the subsequent winter the damage from this malady in mid-western onion-growing districts was of such magnitude as to leave no doubt as to its importance. Investigations were begun in 1917 upon the nature and causes of the disease and possible remedial measures. About the time this work was started, Munn (*20*)[2] published his report in which the cause of neck rot of onion was attributed to *Botrytis allii* Munn. His findings, in so far as they relate to the cause of the disease, have in the main been confirmed. In addition it has been found that a second species, *B. byssoidea* Walker is the more common cause of neck rot in Wisconsin and Illinois sections, and that a third species, *B. squamosa* Walker also causes a neck-rot decay (*34*).

It is the purpose of the present paper to review briefly the history of neck rot as so far treated in the literature, and to report studies upon the three forms of neck rot which are now known to occur. Since each of these three forms produces distinct symptoms and is caused by a distinct species of Botrytis, the three maladies must in any critical consideration be regarded as separate diseases. On the other hand, their similarity in so many respects is justification for referring to them by a group name which will indicate that they are similar diseases caused by closely related organisms. There are several reasons for allowing the term "onion neck rot" to apply to the group. It is an accurate and adequate common term already in good usage; it was probably applied to one or all of the three diseases before any clear differentiation was made; and all three parasites invade the onion bulb chiefly through the neck tissues.

For differentiating between the three types of neck rot it has been suggested that descriptive adjectives be used (*34*). For the form already described by Munn (*20*) as due to *Botrytis allii*, the term "gray-mold neck rot" is used because of the prompt and abundant production of gray conidial masses upon the decaying host tissue. For the second form, due to *B. byssoidea*, the name "mycelial neck rot" is suggested because of the predominance of mycelium and the

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[2] Reference is made by number (italic) to "Literature cited," p. 926.

scarcity of conidia on the decayed host tissue, as compared with the previous form. For the third form, due to *B. squamosa*, the name "small sclerotial neck rot" is suggested because the appearance of small sclerotia upon affected scales (i. e., small as compared with those of the other two forms) is an early and fairly constant sign of the disease.

HISTORY AND DISTRIBUTION OF ONION NECK-ROT DISEASES

The first description of onion neck rot was apparently that by Sorauer in Germany in 1876 (*25*). He again described it in the second edition of his textbook, in 1886 (*26, p. 294–297*). The symptoms which he has recorded correspond closely with those later given by Munn (*20*). He considered the form of Botrytis causing the disease to be *Botrytis cana* (Pers.) Fr. and the sclerotia probably to be identical with those which had been previously described as *Sclerotium cepae* Berk. and Br. He inclined to the view that the latter would give rise to an ascigerous stage identical with *Sclerotinia fuckeliana* but did not adduce any evidence to substantiate this view. It is likely, considering the size of conidia which he reports, that the form he had under observation was identical with *B. allii* Munn. He was able to reproduce the disease by artificial inoculation of healthy bulbs with conidia, but noted that the success of such inoculations depended on the presence of humid conditions. The severity of the disease in the field was dependent to a marked degree upon the amount of humid weather which prevailed about harvest time, a factor which later workers have likewise found to be of great importance. He was unable to produce infection upon healthy, vigorous, green onion leaves. He noted the conspicuous varietal differences in susceptibility, which have been recorded many times since, namely, that the white type of onion is very susceptible, whereas the yellow and red types are much less seriously affected.

Frank (*8*) described the disease, also from Germany, in his textbook in 1880. He added little to the facts given by Sorauer except that he claimed to have produced infection upon the green leaves of onion by means of artificial inoculation. He considered the causal organism to be identical with *Botrytis cinerea* Fr. Smith (*24*) in 1900 discussed the onion neck-rot disease which he found at Munich, Germany, and pointed out that the organism was distinct from *B. cinerea* both with respect to the shape of conidia and with regard to the low, creeping growth of the fungus as it sporulated upon decaying onion tissue. He was most likely working with *B. allii*.

A neck-rot disease was first reported from England by Massee (*18*) in 1894. Though he attributed the cause to *Sclerotinia bulborum* Wakker, experimental proof of the causal relations is lacking. An illustration showing the disease, in a later publication (*19*), indicates that he was dealing with either *Botrytis allii* or *B. byssoidea*. Cotton and Owen (*7*) later reported the occurrence of *B. allii* in England.

In 1903 Voglino (*29*) distinguished the Botrytis disease of onion found in Italy from that caused by *Sclerotium cepivorum* Berk. and referred the neck-rot fungus to *S. ambiguum* Duby.

Hanzawa (*10*) in 1914 reported a Botrytis disease of onion bulbs in Japan. He gave the size of conidia as 8.4 to 16.8 by 6.3 to 10.5 μ and considered that it was probably identical with *Botrytis cinerea*. These measurements correspond most closely with those of *B. byssoidea*, but it is impossible to determine with certainty from his meager description whether the form he studied was *B. allii* or *B. byssoidea*.

Halsted (*9*), the first to record the disease in America, gave a brief report of its occurrence in New Jersey in 1890, and referred to the causal organism as *Botrytis parasitica* Cav. Clinton (*4*, *5*) reported serious epiphytotics of neck rot upon white onions in Connecticut in 1902 and 1903. He reported that the fungus caused yellowish spots on the leaves of the onion plant in July and also blasted the flowers of the seed plant. He pointed out the importance of humid weather in bringing about heavy infection and attributed the absence of the disease in 1904 largely to the dry weather which prevailed during July and August of that year.

Selby (*23*), writing in 1910, considered this to be the most serious disease of white onions in Ohio. He erroneously identified the causal agent as *Sclerotium cepivorum*. This fungus, described originally by Berkeley (*1*), produces minute sclerotia, while the diseased specimens illustrated by Selby are characteristic of those produced by *Botrytis allii* and by *B. byssoidea*. Humbert (*13*), writing on the same disease from Ohio, also referred to the causal organism as *S. cepivorum*, but that this interpretation is erroneous is obvious from his description of conidia and his illustration of a diseased onion bulb, which is similar to that published by Selby.

A neck-rot disease of onion was destructive to onions in Oregon in 1912, according to Jackson (*14*), and from his description it is apparent that *Botrytis allii* was the chief causal agent in that case. Munn (*20*) found neck rot repeatedly in New York and Michigan from 1913 to 1916, and described the disease as caused by *B. allii*. The data accumulated by the Plant Disease Survey (*3*, *6*, *11*, *15*, *28*) from 1921 to 1925 are sufficient to show that onion neck rot is widespread in this country. Although more consistent losses are perhaps sustained in the North, most onion-growing areas suffer from time to time, while the damage in a given locality varies widely with the season.

In many of the references cited above it is hardly possible to distinguish between the three types of neck rot. In practically all of the instances noted it is quite evident that the causal organism was either *Botrytis allii* or *B. byssoidea*. *B. squamosa* seems heretofore to have attracted little attention. *B. allii* has been isolated by the writer from bulbs grown in California, midwestern States, Pennsylvania, and Connecticut, and Munn (*20*) obtained it from Michigan and New York bulbs. Moreover a culture isolated from onions in France and forwarded to the writer by the Centralstelle für Pilzkulturen at Baarn, Holland, under the name of *B. cana* was found to be identical with *B. allii*. The mycelial neck-rot form, *B. byssoidea*, has been isolated from bulbs grown in midwestern States and in Connecticut, and was found by the writer on onions in the market at Paris, France, in 1922. It would appear, therefore, to be quite as widespread in its occurrence as *B. allii*. The small sclerotial

form, *B. squamosa*, has so far been isolated by the writer only from onions grown in the Middle West, but no extensive search has been made for it elsewhere.

BOTRYTIS AND SCLEROTIUM FORMS PREVIOUSLY DESCRIBED ON ALLIUM

The Botrytis and Sclerotium forms upon species of Allium which have been described in literature present a very confusing situation to the reader. Many of these are the result of collections made on dying or dead parts and may or may not have a connection with the neck-rot forms. One of the first sclerotial forms on *Allium cepa* to be described in Europe was *Sclerotium cepivorum* Berkeley (*1*). A recent survey has shown this to be widespread in Europe and to occur in America (*31*). There should be no cause for confusing it with the Botrytis forms on onion for it lacks conidia and has much smaller sclerotia, but nevertheless it has been confused with these forms by Sorauer (*26*), Bruck (*2*), Selby (*23*), and Humbert (*13*). Massee (*18*) refers to the form associated with neck rot as identical with *Sclerotinia bulborum* Wakker on hyacinths, but there seems to be no justification for his statement. Voglino's reference (*29*) of the Botrytis found on *Allium cepa* to *Sclerotium ambiguum* Duby seems unwarranted. The identity of the neck-rot Botrytis with *Botrytis cinerea* has been suggested by Frank (*8*) and Hanzawa (*10*), but the differentiation between the latter and the onion form from Germany with which he worked was pointed out by Smith (*24*), and the distinction between *B. cinerea* and *B. allii* was later made by Munn (*20*). There is no ground for considering either of the Botrytis forms on onion identical with *B. cana*, as suggested by Sorauer (*26*), with *B. aclada* Fres. (*21, v. 4, p. 131*), with *B. fulva* Link (*16, Abt. 8, p. 280–281*), or with *B. vulgaris* Fr. var. *interrupta* Fr. (*21, v. 4, p. 129*) the last three of which have been found on dead scales or stems of Allium. *B. parasitica* Cav., reported on *Allium ursinum* (*16, Abt. 8, p. 292*), and referred to by Halsted (*9*) as the cause of onion neck rot, has been studied recently by Hopkins (*12*) and is undoubtedly distinct from all the onion forms.

Saccardo (*21*) describes several species of Sclerotium which have been found on living or dead parts of various species of Allium. It is not likely that any of these forms are identical with either of the sclerotial forms associated with onion neck rot. *Sclerotium ambiguum* Duby, *S. inconspicuum* Lib., *S. pulveraceum* Dur. and Mont., and *S. sepivorum* Berk. (*21, v. 14, p. 1150–1151*) are all described as having very small sclerotia, smaller than those of *Botrytis squamosa*. A form producing thin, ovate-oblong, minute sclerotia, described as occurring on *Allium vineale*, suggests some similarity to *B. squamosa*, but this form was ascribed to *S. tulipae* Lib. var. *hyacinthi* Guep. (*21, v. 14, p. 1172*), which has been described fully by Hopkins (*12*) and presents no close similarity to the onion neck-rot forms. *S. brassicae* Pers. (*21, v. 14, p. 1164–1165*), and *S. durum* Pers. (*16, Abt. 9, p. 674–675*) are described as producing very large sclerotia. The former, found on *Allium victorialis*, yielded no Botrytis form, while the latter was said to produce a Botrytis stage identical with *B. cinerea*.

An undescribed species of yellow-spored form of Aspergillus was isolated from Italian-grown garlic by E. D. Eddy in 1919. This

species was found by the writer to be pathogenic upon onion bulbs as well as upon garlic. It produces in the decayed bulb tissue dark brown to black, slightly elongate sclerotia 1 to 2 mm. in length, which might be confused with other forms. The yellow color of the spores and the brown cast of the sclerotia are the chief characters by which it is readily distinguished from the above-mentioned sclerotial forms on onion. The causal organism has been recently described as *Aspergillus alliaceus* Thom and Church (27).

Sclerotium rolfsii is sometimes found on bulbs of garlic, but as yet no occurrence on onion bulbs has come to the writer's attention. The sclerotia are readily distinguished from those of the above form by their decidedly lighter brown color.

Sawada (*22, p. 206–209*) has described a disease of *Allium cepa* and *Allium fistulosum* which occurs in Japan and Formosa. This is attributed to a newly described species, *Sclerotinia allii* Sawada. The malady is one which attacks the leaves and stems of the growing plant, however, and is not reported as a storage decay of bulbs. Sawada states that *Sclerotinia libertiana* Fuckl. (*Sclerotinia sclerotiorum* (Lib.) Massee) also attacks onion plants in Japan, but so far as the writer is aware this fungus has not been authentically described elsewhere as an onion pathogene.

GRAY-MOLD NECK ROT

SYMPTOMS

Gray-mold neck rot is found most commonly upon the bulbs after harvest, infection taking place most readily through the neck tissues. The first sign of the disease is the softening of the affected scale tissue, which takes on a sunken, cooked appearance characteristic of the advancing zone of the diseased areas. The margin between the diseased and healthy tissue is quite definite, and since the effect upon the host cells is produced somewhat in advance of the hyphae, usually little evidence of the fungus is visible there. As the mycelium multiplies in the older diseased area the affected tissue becomes grayish in color, and later a dense grayish mycelial mat often develops upon the surface of the scale. Under average conditions, conidial production is prompt. A dense layer of gray mold consisting of comparatively short conidiophores and myriads of conidia is produced on the outer diseased scales. The early production of spores, even under average room conditions, is characteristic of this form, whereas the other two forms are more tardy in sporulating.

The disease progresses most rapidly down the scales which have been originally infected in the neck, while the spread from one scale to another proceeds somewhat more slowly. In a cross section of a partially rotted bulb, at the advance margin of the disease, the parenchyma has a distinctly water-soaked appearance. A few millimeters above this point a grayish tinge and slight shrinkage in the decaying tissue become evident, while still farther up the shrinkage of the scales and the development of mycelium increase. On the outer scales conidial production is pronounced. Sclerotia occur in connection with the older decayed tissue, first as whitish mycelial masses, which become darker with age until they assume the appearance of hard, black, rounded, kernel-like bodies, spherical, oblong, or irregular, and

varying from 1 to 3 mm. or more in length. They form usually on the outer surface of the scale or are slightly embedded in the diseased host tissue.

Occasionally infection takes place through the bases of onion scales or through wounds. The results—the progress of the disease and the appearance of the tissue—in such cases are quite similar to those in cases of neck infection. The older decayed bulb presents the appearance of a "mummy." While the host tissue in the beginning of its decay is somewhat watery, it desiccates rather promptly and does not give the appearance of being affected by common bacterial soft rot. The moisture which is released is often sufficient, however, to stimulate growth of the central bud, and the premature sprouting of affected bulbs is a common sign of the disease. Little or no offensive odor is occasioned by neck-rot decay. Commonly, however, soft-rot bacteria follow close upon *Botrytis allii*, in which case a more watery decay and more odor result. Certain phases of the disease are illustrated in Figure 1.

Botrytis allii, being a faculative saprophyte, develops commonly upon dead onion parts and other refuse in the field. Munn (*20*) reports finding it also upon the roots, leaves, and flower parts of the onion plant. It would appear from his experiments that the organism may act as a parasite upon the aerial parts of the growing plant. The writer has never found any of the three Botrytis forms attacking the actively growing parts of the plant. The older dying leaves and the tips of leaves turning yellow from other cause often show Botrytis conidial production during moist weather, but the actual initial damage caused by Botrytis is not ordinarily noticeable under midwestern conditions.

THE CAUSAL ORGANISM (BOTRYTIS ALLII)

The septate, branching mycelium of *Botrytis allii* has little in the way of distinctive characters. It is hyaline for the most part, sometimes taking on a slight tinge of color with age. The aerial hypae en masse have a smoky-gray cast, but only in the conidiophores do the walls take on a deep-brown color.

Appressoria, or holdfasts, are commonly formed by repeated branching of hyphae which comes into contact with the host surface or with the glass containers of pure cultures. The mycelium in these bodies for the most part contains denser cytoplasm than is found elsewhere, and the walls commonly are darkened in color, giving often a very dark macroscopic appearance to the holdfasts.

Sclerotia are commonly formed in or upon the decayed host tissue. The conditions which promote the formation of these resting bodies have not been worked out. It is not uncommon for an onion bulb to suffer complete decay without the production of sclerotia, but usually abundant sporulation takes place. Newly formed sclerotia appear as white, dense, velvety masses of mycelium, which soon darken somewhat. As development continues the surface assumes a waxy appearance, light brown at first, gradually becoming darker, until a hard, black, kernellike mass results. The mature sclerotia are variously shaped; they are rounded on the upper surface and are either flat or concavely depressed on the lower side when attached to the outer surface of the onion scale. They vary

in size from 1 mm. or less to 4 or 5 mm., but most of them measure from 2 to 4 mm. It is not uncommon, however, to find many sclerotia aggregated into a crusty mass of considerable size. In

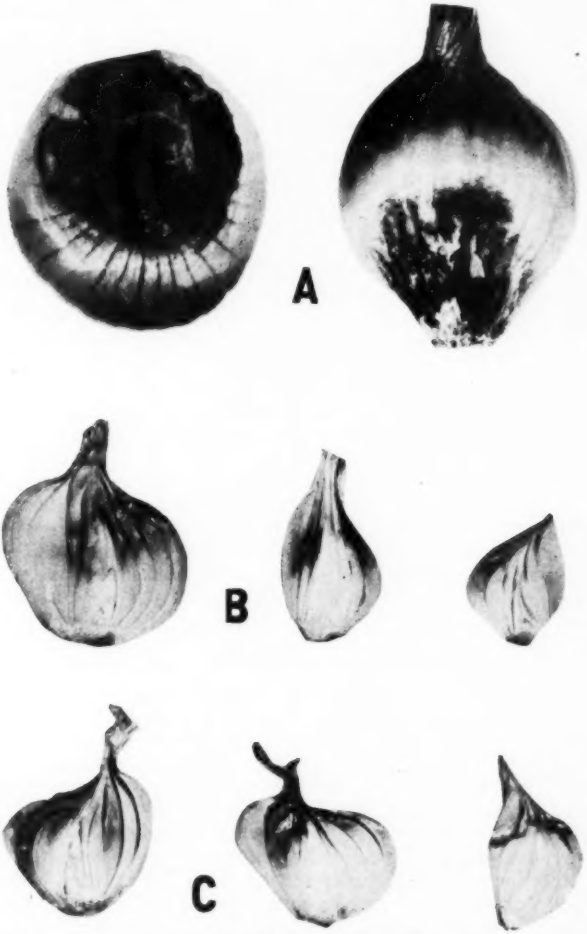

FIG. 1.—A, gray-mold neck rot (*Botrytis allii*). Natural infection. At left, Crystal Wax variety showing neck infection; at right, White Globe variety showing infection through base of bulb. Note characteristic gray mold on the decayed tissue. B, Crystal Wax bulbs inoculated with *Botrytis allii* through needle wounds, placed in moist chambers, and incubated (left to right) at 18°, 23°, and 26° C., respectively, for eight days. C, Crystal Wax bulbs inoculated with *Botrytis byssoidea* but handled otherwise like those in B. Note in B and C that the decay was most rapid at 18° C., somewhat less rapid at 23°, and very meager at 26°

cross section they exhibit a pseudoparenchymatous structure consisting of closely packed mycelial threads. The several outer layers consist of dark-walled cells which give the external black color to

the bodies; the remaining interior is made up of hyaline cells. Under suitable conditions the sclerotia germinate either by sending out hyaline mycelial branches or by the direct production of conidiophores upon their surfaces.

The conidiophores are produced directly from the free mycelium in the tissues or from sclerotia. Branches are sent up approximately at right angles to the mycelial thread. (Fig. 6, A.) These are hyaline at first and remain so in the region of the growing tip, but the older portions of the conidiophore walls become gradually darker until they are a deep brown. They vary from 6 μ to 20 μ or more in diameter, are septate and occasionally branched, and usually attain a fairly uniform height above the surface of the host. With their profuse production of spores, they give a low, dense, gray-mold appearance, often distinguishable from the higher and less-compact habit of *Botrytis byssoidea*. The growing tip of the conidiophores preliminary to spore production sends out several side branches from the main stem and these rebranch once or twice. (Fig. 6, B.) The ends of these branches become rather swollen, and from these rounded tips the sterigmata which later produce the conidia are sent out in large numbers. (Fig. 6, C, D.) These sterigmata, after attaining a length of 1 μ to 2 μ, swell at their tips to form the spores. This takes place almost simultaneously on all the sporiferous tips. (Fig. 6, E.) While the spores are enlarging they are not readily detached from the conidiophore, but when they are mature they disperse readily when placed in liquid or when exposed to air currents. Cleavage of the spore usually takes place at the top of the sterigma, and the sterigma is not usually attached to the spore after dispersal. About the time of spore maturation, cross walls are laid down, first just behind the sporulating tips and finally in close proximity to the main stalk of the conidiophore. Degeneration begins above these septa, starting with the sterigmata and continuing with the sporiferous branches. (Fig. 6, F.)

The main branch of the conidiophore proliferates by extending upward (fig. 6, G), and in due time it again branches and repeats the process of sporulation. Each point of sporulation on the main stalk is marked by slight scars or knobs on the conidiophore where the septa were laid down to cut off the degenerating sporulating branches. (Fig. 6, H.) By this time the walls have usually become dark colored. The conidiophore may thus sporulate and proliferate several times during the course of its development, and the region of sporulation is marked each time by the group of scars where the branches were cut off, while the conidia remain grouped around these points if no liquid chances to come in contact with them or if they are not blown away by air currents. With age the conidiophore becomes flattened and twisted. Thus in nature it is not uncommon to find conidiophores 1 or 2 mm. in length with groups of spores adhering to them at regular intervals. Occasionally one of the sporulating branches does not degenerate but also proliferates and continues to sporulate, thus giving rise to the occasional branch found with the conidiophores of *Botrytis allii*.

In describing this phase of the morphology of the organism, Munn (*20*) does not mention the branching of the conidiophore previous to sporulation, and in describing and figuring the scars upon

the main stalk he intimates that the conidia are produced directly upon them. It is not always easy to demonstrate the exact origin of the conidia, chiefly because the mature conidia disperse as soon as placed in water mount, but after repeated examination of the sporulating tips before the spores are mature and thus not readily detached, there is no reason to doubt that they are always formed in the manner just described.

The conidia are oblong to elliptical in shape, hyaline, and continuous. (Fig. 6, I.) They vary in size over a considerable range (6 to 16 by 4 to 8 μ), but measurement of a considerable number shows that a majority fall within the range of 7 to 11 by 5 to 6 μ. The frequency distribution of length and width for a number of strains growing on onion tissue and on potato agar, as given in Table 1, show a reasonable degree of constancy in range of size. By comparison with measurements of several strains of *Botrytis byssoidea* and of *B. squamosa* given in the same table, it is evident that *B. allii* falls in a class the average measurements of which are somewhat smaller than those of the other two species which affect onion bulbs. Germination takes place under favorable conditions in 12 to 14 hours by the production usually of a single germ tube, occasionally of two, and almost always without septation in the spore during germination. Microconidia are not common; they are globose, about 3 μ in diameter, and are borne on short hyaline conidiophores.

TABLE 1.—*Conidial measurements from a number of strains of Botrytis allii, B. byssoidea, and B. squamosa* [a]

Species	Strain No.	Source	Substrate	Percentage of spores falling within the length (in microns) of groups indicated																		
				6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Botrytis allii	64	Louisiana	Potato agar		10	32	34	16	6	2												
			Onion			12	36	44	8													
	65	(b)	Potato agar		10	26	28	24	6	4		2										
			Onion		2	16	36	36	10													
	76	Illinois	Potato agar	2	10	14	14	22	10	14	4	4	2	4								
			Onion			4	38	40	10	4	2	2										
	77	do	Potato agar		8	14	22	30	16	2	4	4										
	83	Ohio	do		12	22	24	28	10	2	2											
			Onion			18	56	20	6													
	86	do	Potato agar		4	36	30	16	12	2												
	88	Pennsylvania	do	2	20	24	26	20	4	2	2											
	96	Illinois	Onion				32	40	20	4	4											
	100	California	do	12	36	44	8															
Botrytis byssoidea.	42	Wisconsin	do			2	2	4	2	12	12	30	22	2	8	2	2					
	70	Illinois	do					2	6	10	28	28	14	4	2	6						
	80	Wisconsin	do					4	12	26	14	20	12	2	4	2	2	2				
	89	Illinois	Potato agar				2	30	22	20	20	6										
			Onion			6	8	50	30	6												
	90–1	do	Potato agar				6	30	44	16	2	0	0	2								
	90–5	do	do				6	6	20	34	16	12	6									
	90–7	do	do				2	10	16	26	16	14	6	8	2							
	98	Wisconsin	Onion leaf						20	52	12	8	8									
	101	do	do					4	36	16	44											
	114	France	Onion			2	5	17	45	23	7	1										
	94	Illinois	do						4	32	20	28	12		4							
Botrytis squamosa.	27	Wisconsin	Potato agar								2	6	16	20	18	12	20	6				
	30	do	do									2		8	6	8	22	18	18	12	6	
	134	do	do									3	4	15	15	17	17	11	7	9	1	1

[a] In most cases 100 spores from each strain were measured.
[b] Culture No. 65 was furnished by M. T. Munn, New York State Agricultural Experiment Station.

TABLE 1.—*Conidial measurements from a number of strains of Botrytis allii, B. byssoidea, and B. squamosa*—Continued

Species	Strain No.	Source	Substrate	Percentage of spores falling within the width (in microns) of groups indicated													
				4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Botrytis allii	64	Louisiana	Potato agar		58	40	2											
			Onion		18	66	16											
	65	(a)	Potato agar	2	88	10												
			Onion		6	42	42	10										
	76	Illinois	Potato agar	4	68	26	2											
			Onion		18	60	20	2										
	77	do	Potato agar	6	80	14												
	83	Ohio	do		64	36												
			Onion		38	60	2											
	86	do	Potato agar	4	58	36	2											
	88	Pennsylvania	do		62	38												
	96	Illinois	Onion		24	68	8											
	100	California	do	36	48	16												
Botrytis byssoidea	42	Wisconsin	do		2		26	56	14	2								
	70	Illinois	do			6	24	28	32	10								
	80	Wisconsin	do		4	6	34	36	18	2								
	89	Illinois	Potato agar			6	28	32	26	8								
			Onion		2	28	62	8										
	90-1	do	Potato agar				68	30	2									
	90-5	do	do				24	60	14	2								
	90-7	do	do			6	38	26	12	4	4							
	98	Wisconsin	Onion leaf			8	60	28	4									
	101	do	do		24	28	48											
	114	France	Onion			17	74	9										
	94	Illinois	do			20	44	32	4									
Botrytis squamosa	27	Wisconsin	Potato agar						2		12	38	24	22	2			
	30	do	do							2		4	22	36	24	10	2	
	134	do	do						3	8	30	29	9	10	7	3		1

[a] Culture No. 65 was furnished by M. T. Munn, New York State Agricultural Experiment Station.

PATHOGENICITY

Munn (*20*) has already given conclusive proof of the pathogenicity of *Botrytis allii* upon onion bulbs. Many experiments have been performed by the writer in which the conidia or mycelium have been injected into wounds in the succulent scales. In general, when too rapid desiccation of the wounds was prevented and the bulbs were kept at any temperature between 5° and 20° C. positive infection resulted uniformly. The most satisfactory method of obtaining infection has been to inject conidia into needle wounds, placing bulbs in oiled paper bags and incubating them at 15° to 20°. Infection of bulbs by spraying spores on the unwounded surfaces of dry or succulent bulb scales was found by Munn (*20*) to be difficult to attain. The writer has likewise failed to produce infection by this means. It is evident, however, that the fungus may penetrate the unbroken cuticle of succulent scales. This was demonstrated by carefully inoculating the outer succulent scale of a bulb in the usual manner. After the resulting lesion had advanced to a certain point the outer scale was removed, and invasion of the adjacent underlying scale was found to have occurred. This experiment was repeated several times with uniform results. From this fact it is evident that when natural infection of a single scale takes place at the neck or through wounds at other points on the bulb the fungus is not necessarily confined to the scale originally infected. It is true that the fungus does spread more rapidly through the tissue of the first scale infected, but

advance into adjoining scales is not precluded. However, initial infection through the unwounded outer scales apparently does not occur, or in any case is exceptional. When inoculation is made in the succulent tissue, the fungus decays the bulbs of colored and white varieties equally well. The nature of resistance of colored bulbs is discussed later in this paper.

When the onion plant is mature, infection may occur through the unwounded neck tissues, most of which by that time are dead or senescent. This has been shown by artificially inoculating bulbs with and without tops. For further discussion of these experiments the reader is referred to a subsequent section on "Relation of stage of maturity of host to infection of bulbs."

Munn (*20*) produced infection repeatedly by applying spores to the green leaves of plants in a humid atmosphere. Two experiments were performed by the writer in which spores were applied to leaves of vigorously growing plants which were then placed in a moist chamber at about 18° C. for 48 hours. Fine droplets of water covered the plants during this period, and conditions should have been ideal for infection. The plants were removed from the chamber to a greenhouse in which the temperature ranged from 15° to 20°. No infection became evident in either experiment. Several experiments were then performed in which the plants were left in the moist chamber for longer periods. After the first week the infection of some of the leaves was evidenced by the softening of the affected tissue, and this softening was followed by the characteristic sporulation of the organism on the decayed areas. Under these conditions, results quite similar to those of Munn were obtained. It seems to be necessary, however, to keep the plants in a very humid environment for fairly long periods in order to produce infection. When affected plants were removed to ordinary greenhouse environment, the progress of the fungus was almost, if not entirely, checked. The writer is inclined to consider the disease of the aerial parts other than the bulb caused by this fungus as a very mild type of parasitism, which takes place only under extreme and abnormal conditions. This evidence, together with field observations, would lead one to conclude that *Botrytis allii* is of strictly limited importance as a parasite of the aerial portions of the plant. Under extremely humid conditions it might cause slight damage, but how much of the action was truly parasitic and how much was mere subsistence upon senescent or dead tissue would remain an open question. Its occurrence on senescent or dead tissues is no doubt common, and its development upon dead leaves and dead tips of leaves during the growing season of the host is undoubtedly an important part of the life cycle of the oragnism. That the occurrence of Botrytis apparently damaged onion leaves has been reported in a few instances (*4*, *5*, *11*, *17*, *20*).

HOST RANGE

By artificial inoculation, bulbs of white multiplier onion (*Allium cepa* var. *bulbellifera*) and white shallot (*A. ascalonicum*) were found to be quite as susceptible to gray-mold neck rot as those of common onion. Garlic bulbs (*A. sativum*) were also readily infected, but the progress of the disease was much slower on these than on either of the hosts just mentioned.

MYCELIAL NECK ROT

SYMPTOMS

In the early stages of its development the mycelial neck rot is not readily distinguishable from the gray-mold neck rot. As in the latter, the usual avenue of infection is through the neck tissues, but occasionally cases are found where the invasion takes place by way of the stem plate or through wounds at other points upon the onion scales. The same water-soaked, sunken appearance typifies the newly infected tissue, and the advancing margins of the diseased area continue to show this symptom. The line of demarcation between healthy and diseased tissue is also usually well defined, and as in the case of *Botrytis allii*, the mycelial neck-rot organism initiates the breakdown of the host tissue somewhat in advance of the hyphae. In the older decayed parts the scales become shrunken, and grayish superficial mycelium develops in a manner not unlike that of *B. allii*. There is in the main, however, a greater quantity of superficial mycelium formed in the case of mycelial neck rot than would be the case with gray-mold neck rot under like environment. Recently inoculated bulbs of the two forms placed in moist chambers respond quite differently. In the case of gray-mold neck rot the organism sporulates promptly, with little additional superficial mycelium, while *B. byssoidea* usually produces a profuse quantity of mycelium, white at first but later turning gray with age. The most striking point of difference between the two forms is that the mycelial neck-rot organism sporulates very sparsely in these early stages, and it is often difficult to bring about conidial production on newly decaying bulbs even when the latter are placed in the moist chamber. As the mycelial neck rot progresses sclerotia commonly develop in the older decayed tissue. They resemble very closely those of *B. allii* in all respects. Sporulation occurs to some degree under sufficiently moist conditions on the older decayed tissue. The sporulating mass is light-gray at first, gradually becoming darker with age, the darker shade being due in the main to the wall coloration in the older portions of the conidiophores. As a rule the sporulating mass is distinguishable from that of *B. allii* because of the fact that the conidiophores of the latter are usually somewhat shorter, less branched, and more prolific, giving the appearance of a low dense mass of conidia. In the case of *B. byssoidea*, on the other hand, the conidiophores assume somewhat greater length, are more branched, and sporulate less profusely, giving in the end the appearance of a more fluffy development of aerial mycelium with a less dense conidial mass.

It will be seen from this description that points of macroscopic difference between the symptoms of the two diseases are not sufficiently marked to afford even the trained casual observer a ready means of distinction. The outstanding macroscopic distinguishing characters which one may use with reasonable accuracy after some experience in observing the two diseases are (1) the comparative lack of sporulation in mycelial neck rot and the abundance of sporulation in gray-mold neck rot, and (2) the fact that when sporulation does occur in mycelial neck rot, the ranker growth of conidiophores is usually noticeably different from the low dense growth of the sporulating hyphae of the gray-mold neck rot. Distinction is made the more difficult by the fact that at times the two forms may be found

together upon the same bulb. Very often one who is work ng constantly with the two diseases finds it necessary to resort to artificial culturing of the organisms to make diagnosis certain. Growing the organisms upon potato-dextrose agar is a ready means of distinguishing between them. (See fig. 5.) *Botrytis allii* will invariably sporulate abundantly with a few days, while the mycelial neck-rot organism produces rank-growing, fluffy, white aerial mycelium, with seldom, if ever, any evidence of sporulation. Certain aspects of the disease are shown in Figures 2 and 3.

THE CAUSAL ORGANISM (BOTRYTIS BYSSOIDEA)

The mycelium of *Botrytis byssoidea* has no characters by which it may be readily distinguished from *B. allii*. The appressoria or holdfasts are also very similar in the two species. Sclerotia are formed in the same manner and are much alike as to size, shape, and color.

The conidiophores of *Botrytis byssoidea*, like those of *B. allii*, originate either from the free mycelium or from the sclerotia, and their development and the method of spore production in the two forms is of the same general type. A few points of difference in detail are to be noted. Conidiophores are produced much less commonly on the decayed host tissue in *B. byssoidea*, and they are seldom found on artificial media. When produced upon decaying onion scales they are noticeably more scattered than the conidiophores of *B. allii;* permanent branches are formed a little more commonly; and the production of spores is not so profuse. The conidiophores are hyaline at the growing tip, and the walls turn brown promptly as they grow older. To the naked eye the fungus as it fruits upon onion tissue presents a less crowded, more cottony, and somewhat more elevated appearance than the low dense gray-mold development of *B. allii*. The fertile tips of the conidiophores branch and produce spores in the same manner as described in connection with *B. allii*. (Fig. 6, M.) The sporiferous side branches usually degenerate as far back as the septum, which is laid down close to the main trunk of the conidiophore (fig. 6, N); occasionally one persists and continues to grow out as a permanent fertile branch. In this form, as in *B. allii*, the branches, whether permanent or not, show a marked constriction at the point of juncture with the main trunk when they have reached their maximum diameter, and in the case of the permanent branch this constriction remains. As in the other forms, it is common for the conidia to adhere to the sides of the main trunk of the conidiophore until they are removed by water or air currents. The scars left by the degenerated fertile branches are quite similar to those of *B. allii*.

The conidia of *Botrytis byssoidea* are very similar to those of *B. allii* in shape, color, and mode of germination. (Fig. 6, O.) They are occasionally a little longer in relation to their width and thus have a more oblong appearance. They vary considerably in size (8 to 20 by 5 to 11 μ), but, as is shown in Table 1 the majority fall within the limits of 10 to 16 by 6 to 8 μ. They therefore will constantly average somewhat larger than those of *B. allii*, both upon onion tissue and when they are occasionally produced upon potato agar. Microconidia, globose and about 3 μ in diameter, are occasionally formed.

FIG. 2.—Comparison of gray-mold and mycelial neck rots. Healthy White Portugal onion sets inoculated after harvest by the injection of spores or mycelium through neck wounds, placed in standard storage crates, and left in an onion-set warehouse at Morton Grove, Ill., for eight weeks. Those in the upper row were inoculated with *Botrytis allii*. Note the characteristic gray mold on the decayed tissue. The lower five bulbs were inoculated with *B. byssoidea*. Note the absence of conidia and the development of aerial mycelium and of sclerotia on the decayed tissue

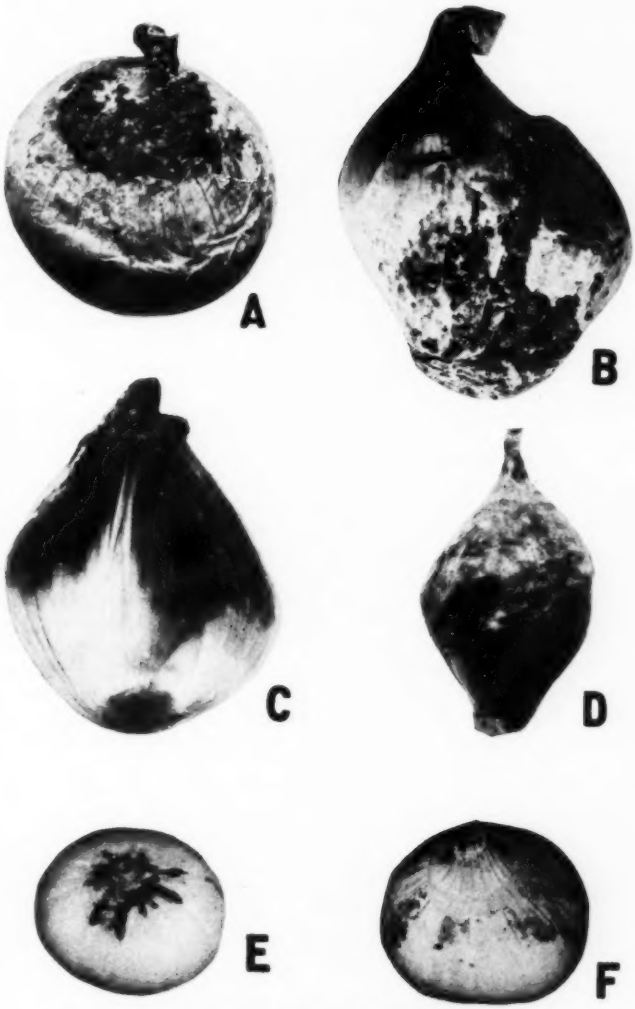

FIG. 3.—Mycelial neck rot *Botrytis byssoidea* on White Globe (A to D) and White Portugal (E, F) onions. Note the formation of sclerotia on the older decayed tissue (A, B). Basal infection commonly occurs (B). The first evidence of infection of the tissue is a water-soaked appearance; the decayed tissue becomes permeated with mycelium and gradually shrivels (C). An abundance of superficial mycelium is common (D). An early stage of infection at the neck is shown in F. Occasionally rapid desiccation of newly infected scales checks the further progress of the fungus (F)

PATHOGENICITY

The mycelial neck-rot disease has been produced repeatedly by inoculating onion bulbs through needle wounds with mycelium from pure culture and with conidia and conidiophores which developed upon naturally infected or upon artificially inoculated bulbs. As in the case of *Botrytis allii*, the most uniform results were obtained when too-rapid desiccation of the wound tissue was prevented. The most satisfactory results were obtained by placing the inoculated bulbs in oiled paper bags and incubating them at 15° to 20° C. Under these conditions the characteristic bulb rot occurred, and the progress of the decay was approximately equal to that of gray-mold neck rot. Under humid conditions and favorable temperature, invasion of the unwounded outer succulent scales has been noted, and the spread from scale to scale through the unbroken cuticle has been demonstrated. Initial infection through the dry outer scales or through the unbroken outer succulent scale does not commonly occur in nature, however, and there is little evidence of natural invasion except by way of wounds or the dying tissues at the neck of the bulbs.

With respect to leaf infection, what has already been said of a gray-mold neck rot may well apply to mycelial neck rot. No evidence has been found in nature that this organism causes a serious blight of onion tops. It is commonly found fruiting upon dead or senescent leaf tissue during humid weather, but is to be regarded as little more than a saprophyte. Vigorously growing onion plants inoculated with a spore suspension of the organism and kept in a moist chamber at about 18° C. for 48 hours and removed to a greenhouse at 15° to 20° did not yield evidence of infection. As in the case of *Botrytis allii*, plants inoculated in such a manner and kept under very humid conditions at 15° to 20° for one to two weeks eventually showed evidence of leaf infection. It is questionable, however, if this situation commonly exists naturally in onion-growing regions in the United States. *B. byssoidea* was found in one instance causing secondary infection of seed stems of Egyptian onion that has been infected with rust (*Puccinia asparagi*) (*30*). In the original report this was not considered to be a neck-rot form, since first inoculations of the bulbs gave negative results. When the proper conditions for infection were later determined, positive infection of bulbs with this strain was obtained.

HOST RANGE

Bulbs of white multiplier onion and of white shallot when inoculated with *Botrytis byssoidea* decayed quite as rapidly as did those of common onion. Bulbs of garlic were also successfully inoculated, but, as in the case of *B. allii*, the progress of the disease was distinctly slower than upon the other hosts.

SMALL SCLEROTIAL NECK ROT

SYMPTOMS

The small sclerotial neck rot has so far, with one exception, been found only upon white varieties of onion. In one instance it was found in a few very slightly pigmented bulbs which appeared in a lot of Yellow Globe variety and were probably the result of previous crossing with a white variety. As will be pointed out later, colored bulbs are

readily infected when inoculated through wounds in the succulent scales. The fungus most commonly appears first near the neck of the bulb and, as a rule, not until some weeks after the crop has been harvested and stored. It is often confined to the two or three dry outer scales. At times it is found upon the succulent scales, commonly in association with the mycelial neck rot.

Usually the first evidence of the disease is the appearance of very thin, scalelike sclerotia which adhere very closely to the dry scales. The sclerotia are at first light colored, but most of them turn completely black with age. In many instances their development is arrested prematurely, and bodies which are light gray in the center and black only at the margin result. The sclerotia are usually roughly circular and measure from ½ to 1½ mm. in diameter. They vary from this form, however, being sometimes larger and more irregular in shape. The mycelium of the fungus is scanty and not often distinguishable microscopically upon the affected dry outer scales. When it does appear it is scattered and has a dark-green cast. Sporulation occurs on conidiophores, arising most commonly from the sclerotia and more rarely from the scattered mycelium. At room temperature very little sporulation can be induced even when affected scales are placed in a moist chamber, but under moist conditions in the ice box or at temperatures between 5° and 18° C. sporulation upon the sclerotia is common.

The decay of the succulent scales is distinctly slower than that caused by either *Botrytis allii* or *B. byssoidea*. The cytolytic action in advance of the hyphae, characteristic of the other two forms, prevails in this case as well. The newly rotted area is water-soaked in appearance, and there is a quite definite margin between diseased and healthy tissue. In the older decayed portions grayish superficial mycelium is formed, though usually rather scantily, while sclerotia are also commonly embedded in the rotted tissue. The decayed portions of the bulb often become distinctly brownish in color, and in this respect the disease is distinct from the gray-mold and the mycelial neck rots. Certain aspects of the disease are illustrated in Figure 4.

THE CAUSAL ORGANISM (BOTRYTIS SQUAMOSA)

The mycelium of the small sclerotial form is not sufficiently different from that of the other two onion Botrytis forms to be of diagnostic value. Appressoria are commonly produced. The sclerotia are quite characteristic. They vary in size from ½ to 1½ mm. or more, and often converge into large, irregular scalelike crusts. On the host they are almost always very thin and flat and seldom acquire the thickness of ½ mm. They are usually roughly circular, but occasionally they are quite irregular in form. They appear first as dense, thin mycelial mats of a dirty-white color. They become darker with age and gradually become black, the color appearing first at the outer margins and progressing inwardly until the whole sclerotium is deep black. Occasionally the development of color is arrested prematurely, with the result that the sclerotia have light grayish centers and black margins.

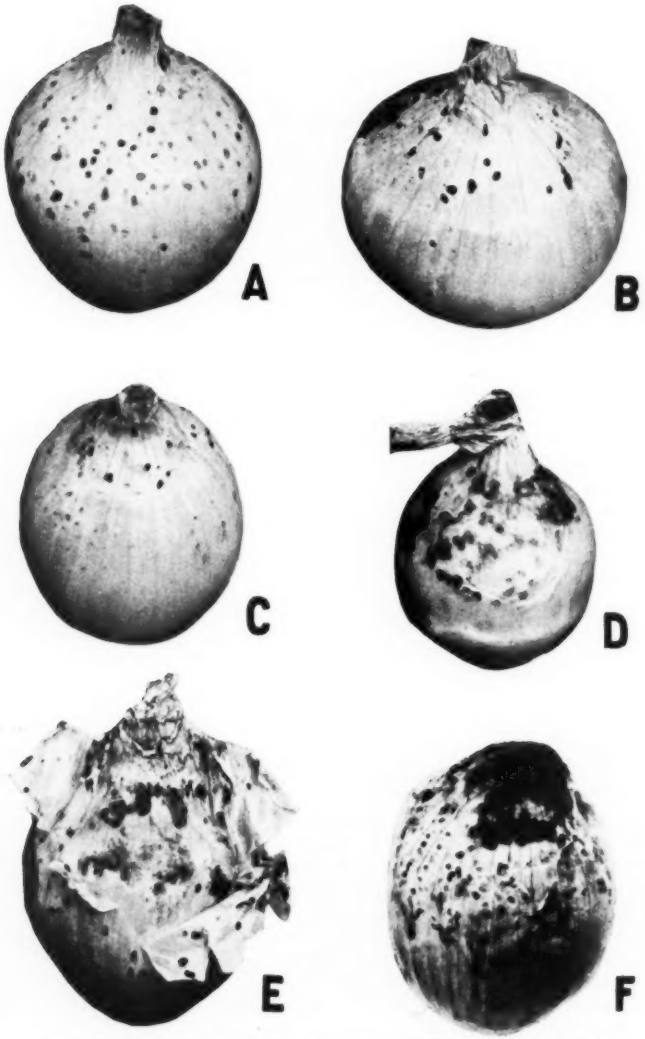

FIG. 4.—Small sclerotial neck rot *Botrytis squamosa* on White Globe onions. Thin, flat, black sclerotia develop commonly on the dry outer scales. Invasion of the succulent scales sometimes occurs (D, E). In F, *Botrytis byssoidea* is also present in the succulent scales. The larger, more rounded sclerotia near the neck of the bulb are those of the latter fungus

The conidiophores arise occasionally directly from free mycelium, but in the main are produced upon the sclerotia. They are hyaline at the growing tip, but the walls turn dark promptly as they become a little older. They are septate, and occasionally branched, the branches being constricted at the point of juncture with the main stalk. Previous to sporulation the growing tip gives rise to a number of branches which rebranch and at their rounded tips send out sterigmata upon which the conidia are borne in the same manner as described for *B. allii* and *B. byssoidea*. (Fig. 6, J.) The sporulating branches are, as a rule, shorter and plumper than those described for the other two forms. As the spores mature, the branches, having been cut off close to the main stalk by septa, rapidly degenerate. As they do so the walls of these branches contract in a unique manner and give the appearance of closing together in folds suggestive of the contracting bellows of a camera. (Fig. 6, K.) The spores, if not removed by air current or by water, adhere in a group to the sides of the main stem of the conidiophore which now proliferates by growing upward, again branching and sporulating. Scars or knobs not unlike those previously described for the other two species mark the points at which degenerating sporiferous branches are attached. Occasionally one of the branches becomes permanent.

The conidia are hyaline, ovoid, and continuous, and germinate within 24 hours under favorable conditions by each sending out usually one hyaline germ tube. (Fig. 6, L.) Septation of the spores upon germination is not common. They vary in size from 13 to 24 μ by 9 to 18 μ, but the majority of them fall within the limits of 15 to 22 by 11 to 15 μ. (See Table 1.) Microconidia, globose and about 3 μ in diameter, are occasionally formed.

PATHOGENICITY

Inoculation of healthy onion bulbs was brought about by injecting mycelium or sclerotia from pure cultures of *Botrytis squamosa* into needle wounds made near the necks. The bulbs were placed in oiled paper bags and incubated at various temperatures. The most rapid decay occurred at about 16° C., although infection did occur over a range of 4° to 22°. Even at the optimum the decay progressed much more slowly than in the case of *B. allii* or *B. byssoidea*. Results were obtained, however, which left no doubt that the organism is pathogenic upon the succulent scales and that under these conditions colored bulbs decayed quite as readily as white bulbs. Direct infection through the unwounded surface of the succulent scale occurs under proper conditions of humidity and temperature, although this does not appear to be the usual method of initial infection.

Vigorously growing onion plants were inoculated with a spore suspension of the organism and transferred at once to a saturated atmosphere held at about 18° C. After 48 hours the plants were transferred to a greenhouse kept at 15° to 20°. No evidence of infection developed. When plants were left in the moist chamber for a week or more, leaves were attacked in the manner already described in connection with *Botrytis allii* and *B. byssoidea*. *B. squamosa* was somewhat more aggressive under these conditions than were the other two species. Severely infected plants, when removed to ordinary greenhouse conditions, rapidly outgrew the disease. It appears, therefore, that in an extremely humid environment having

a favorable temperature, infection of the aerial parts by *B. squamosa* may occur. But, as in the case of the other two neck-rot organisms, there is little evidence that this form causes a destructive leaf blight, and its relation to the host during the growing season is primarily one of subsistence on dead or senescent tissue.

Infection of bulbs through the unwounded neck has not been produced by artificial inoculation, but the common observation of the disease upon bulbs regardless of whether the tops were clipped at harvest shows that the organism penetrates the scales by way of the senescent leaf tissue without the aid of artificial wounds.

COMPARATIVE STUDIES OF THE THREE NECK-ROT ORGANISMS

CULTURAL CHARACTERS

All three species of Botrytis associated with onion neck rot grow readily upon onion and potato-dextrose agar. On either of these media the growth characters of each species are quite distinctive, and they afford a convenient means of differentiation. It has not been found necessary to study the development of these fungi on a wide range of artificial media. Potato agar containing 2 per cent dextrose has been used as stock medium, and the salient characters of growth on this agar will be given.

Botrytis allii (fig. 5, A)

On potato-agar plates a rapidly growing colony was produced, which attained a diameter of about 50 cm. in 4 days at 20° to 22° C. In the beginning the center of the colony consisted of dense whitish aerial mycelium, while the outer zone, about 5 mm. in width, consisted of scanty, creeping, extending mycelial threads. Conidiophores and conidia appeared on about the third day, nearly white at first, turning smoky gray with age. Eventually the entire plate became covered with a dense uniform layer of conidiophores. Sclerotia were not usually produced but occasionally developed in test-tube cultures with this medium. Dark-colored appressoria were sometimes formed when the mycelium penetrated the agar layer and came in contact with the glass plate, but here again the development was more pronounced in test-tube cultures at the edge of the slant. There was little difference in growth to be noted when maltose, lactose, or galactose was substituted for dextrose in the medium. Onion-scale agar and potato agar made without the addition of sugar produced growth quite similar to that on potato-dextrose agar, the chief point of difference being the smaller amount of appressorial development. The organism was carried for seven years on potato-dextrose agar without any noticeable change in growth characters, and this period of artificial culturing did not change the pathogenicity of the fungus.

Botrytis byssoidea (fig. 5, B)

On potato-dextrose agar plates the *Botrytis byssoidea* colony grew somewhat faster at 20° to 22° C. than did *B. allii*. Abundant white, fluffy, aerial mycelium was formed, which was more raised and cottony than that of either *B. allii* or *B. squamosa*. It was fairly uniform in density except at the narrow outer zone of extending

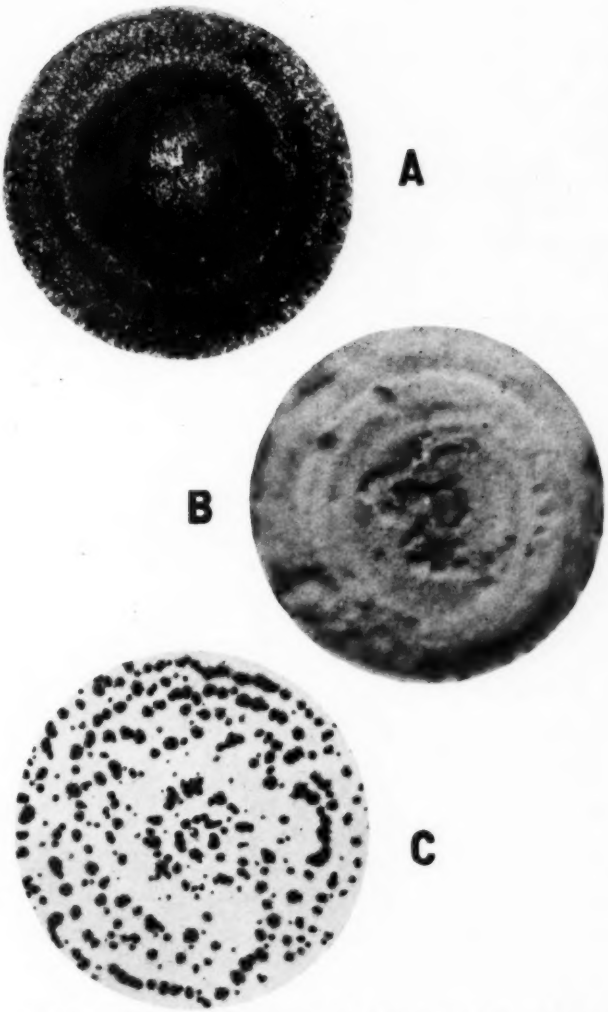

FIG. 5.—The three onion neck-rot organisms on potato-dextrose agar. A, *Botrytis allii*. Profuse production of conidia. B, *Botrytis byssoidea*. Profuse mycelial development but no conidia. C, *Botrytis squamosa*. Mycelial development followed by production of sclerotia. No sporulation occurred at room temperature, 20° to 22° C. After two months the culture was removed to the ice box, where profuse sporulation took place. Note the conidiophores arising from the sclerotia

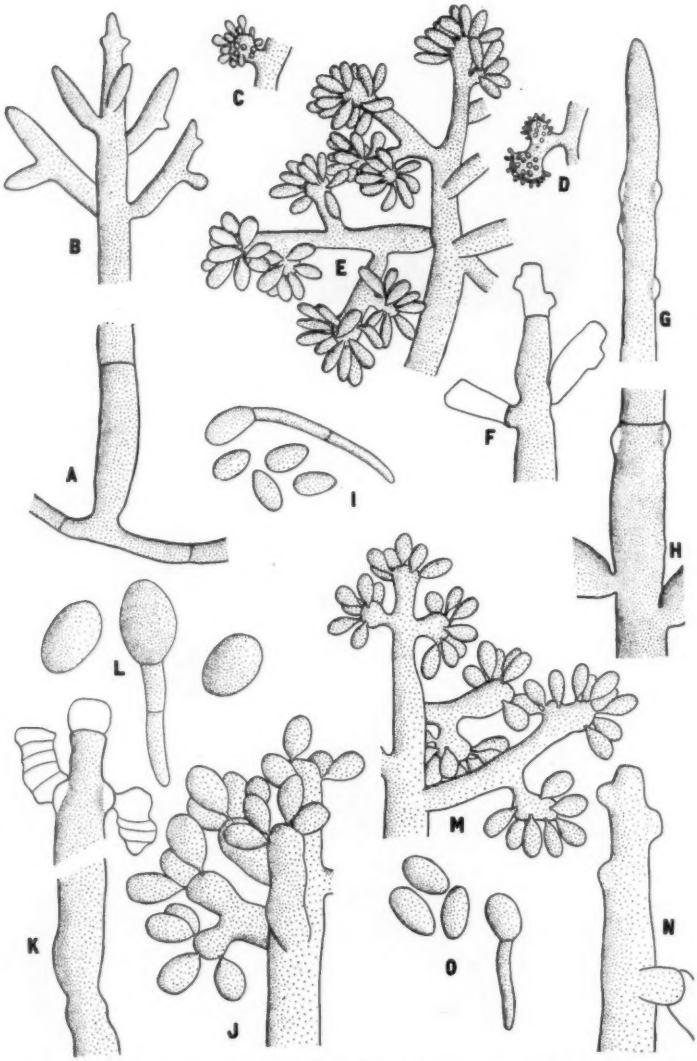

FIG. 6.—Conidial production. A to I, *Botrytis allii;* J to L, *B. squamosa;* M to O, *B. byssoidea.* Conidiophores arise from mycelium (A) and branch previous to sporulation (B). The sporiferous tips become rounded and send out numerous sterigmata (D) from which growth continues (C) to produce conidia (E). When the spores are mature the branches degenerate (F) and the main stalk proliferates (G). Groups of sporiferous branches are thus formed several times leaving scars on the main branch (G) in each case. Sometimes the branches become permanent (H). The process is similar in each species (J, K, and M, N). Note characteristic accordionlike folds in degenerating sporiferous branches of *B. squamosa* (K). See further explanation in text

hyphae. Conidia were seldom produced. Appressorial development was pronounced at the edge of test-tube slant cultures. Sclerotia were usually absent in both plate and tube cultures grown at room temperatures. In young tube cultures placed out of doors on January 25, 1926, at Madison, Wis., numerous characteristic sclerotia had formed when they were brought in on March 19. In another culture, about a month old when placed out of doors on December 5, 1925, and brought in on March 19, 1926, sclerotial formation did not take place but conidial production was quite pronounced. When maltose, lactose, or galactose was substituted for dextrose no conspicuous change in growth characters resulted. On onion agar and potato agar without dextrose the mycelial production was somewhat less profuse. Whereas *B. allii* was carried on potato-dextrose agar indefinitely without loss of pathogenicity, *B. byssoidea* usually lost entirely its pathogenicity within a few months under such treatment, especially when kept at room temperatures (20° to 22°). This change was accompanied by a reduction in the vigor of the culture as exhibited by less profuse aerial mycelium and less appressorial development.

Botrytis squamosa (fig. 5, C)

On potato-agar plates the *Botrytis squamosa* colony enlarged more slowly than either *B. allii* or *B. byssoidea*. It consisted of white, fluffy, aerial mycelium, except for the outer advancing zone, but was less raised and fluffy than *B. byssoidea*. Numerous sclerotia appeared over the entire plate after two to three weeks, first as dense whitish mycelial masses which became hard and black with age. They were mostly 1 to 2 mm. in width and thicker and more rounded on the upper surface than those which developed upon the host. There was very little sporulation at room temperature (20° to 22° C.), but profuse production of conidiophores arising directly from the sclerotia occurred at 12° to 16°. Substituting maltose and lactose for dextrose made little difference in the growth produced. When galactose was used, however, the sclerotia assumed a distinctly different form. They were a little larger, more irregular in shape, very thin and flat, and embedded just at or slightly below the surface of the substrate. On onion agar and potato agar made up without the addition of sugar fewer sclerotia were produced.

Overwintering and Seasonal Cycles

Experiments were undertaken to determine by what means the three neck-rot organisms might live through the winter at Madison, Wis. Sclerotia and spores on bulbs affected by mycelial and gray-mold neck rot were exposed to prevailing weather from December to March in the winter of 1925–26 by placing them in wire baskets on an outer window sill. Both sclerotia and spores of each organism were viable at the end of this period. Pure cultures were exposed over the same period, and they also remained viable. A culture of the small sclerotial neck-rot organism containing sclerotia and conidia was also exposed. Transfers from sclerotia as well as from the conidia and conidiophores were made at the end of the period with positive results in each case. It is thus apparent that the sclerotia will withstand winter weather, while it is not improbable that the mycelium and spores under certain conditions may also live through the extremely cold portion of the year.

From general field observations it is quite evident that each of the organisms persists from year to year in the soil or on refuse. The organisms from such materials are undoubtedly the beginning of saprogenic development the following season, when they live as saprophytes upon dying onion leaves and other organic matter during the growing season of the host. Spores from this saprogenic phase of the organisms are the source of inocula for bulb infection at and after the end of the growing season.

TEMPERATURE RELATIONS

Growth on Media

The relation of temperature to the range and rate of growth of the three organisms was studied upon potato-dextrose agar. Separate slants were inoculated each with one of the three organisms and placed in constant-temperature incubators the temperature of which ranged from 3° to 33° C. The three organisms responded quite similarly to the various temperatures. Growth ensued very slowly at 3° to 4°. There was a gradual increase in the rate of growth with increase in temperature up to about 20° while the rate was about the same between 20° and 25°. At 28° there was decidedly less growth than at the optimum, and at 30° there was still greater retardation. At 33° there was some growth of each organism, but it was very meager in all cases. Thus all three forms grew upon potato-dextrose agar within the range of 3° to 33°, the growth being very slow at either end of the range and most rapid at about 20° to 25 °.

Spore Germination

Germination tests were conducted at various temperatures with the conidia of each of the three species. Spore suspensions were made in tap water and drops transferred to clean glass slides, which were placed in moist chambers in the incubators. In Table 2 the rate of germination in each incubator is given. Spores of *Botrytis squamosa* germinated more promptly than those of the other two species. It is to be seen that germination occurred more promptly at temperatures between 19° and 27° C. than at temperatures below 19°, but, on the other hand, it took place over the entire range of temperature with each species after a period of 24 hours.

TABLE 2.—*Relation of temperature to spore germination[a] of Botrytis allii, B. byssoidea, and B. squamosa*

Temperature °C.	Species and interval											
	Botrytis allii				Botrytis byssoidea				Botrytis squamosa			
	4 hours	8 hours	12 hours	24 hours	4 hours	8 hours	12 hours	24 hours	4 hours	8 hours	12 hours	24 hours
3.5–4	0	0	0	+	0	0	0	+	0	0	0	+
6.5–7	0	0	0	+	0	0	0	+	0	0	0	+
8.5–10	0	0	0	+	0	0	0	+	0	0	0	+
13.5–15	0	0	+	+	0	0	+	+	+	+	+	+
16.5–18	0	0	+	+	0	0	+	+	+	+	+	+
19.5–20	0	+	+	+	0	+	+	+	+	+	+	+
22.5–23	0	+	+	+	0	+	+	+	+	+	+	+
24.5–25.5	0	+	+	+	0	+	+	+	+	+	+	+
26.5–27	0	+	+	+	0	+	+	+	0	+	+	+

[a] + indicates germination; 0 indicates no germination.

Sporulation

It is of course important to know the bearing of temperature upon the production of spores of the fungi when exposed to properly humid conditions. *Botrytis allii* appears to sporulate readily at comparatively low relative humidity, but that the other two forms do is not so evident. *B. allii* sporulates readily in culture or upon rotted onion tissue over a range of 4° to 25° C. *B. byssoidea*, although it does not sporulate readily in culture, will do so when old decayed onion tissue is placed in a moist chamber. Under these conditions *B. byssoidea* produces conidia most quickly at about 23° but quite promptly within a range of 13° to 27°. At temperatures between 3° and 13° it sporulates more slowly. *B. squamosa* has a more limited range of sporulation. No conidia have been found at 22° either in pure cultures or on decayed tissue kept in a moist chamber. The range at which sporulation ordinarily occurs under these conditions is about 18° to 3°. The optimum is about 16°.

Infection and Progress of Decay

A number of experiments were made to determine the relation of temperature to infection and the rate of progress of decay. The bulbs were all inoculated by injecting mycelium or spores into needle wounds near the neck. They were then placed either in oiled paper bags or in uncovered paper boxes and incubated at different temperatures. The first two experiments are concerned with *Botrytis allii* and *B. byssoidea*. In the first experiment, the results of which are given in Table 3, the bulbs were all placed in oiled paper bags.

Table 3.—*The relation of temperature to infection and progress of decay of onion bulbs inoculated with Botrytis allii and B. byssoidea; five bulbs in each lot; Red Wethersfield variety*

Temperature °C.	Botrytis allii		Botrytis byssoidea		Control
	Per cent infected	Rate of progress[a]	Per cent infected	Rate of progress[a]	Per cent infected
2–4	80	1	100	1	0
5–8	100	2	100	2	0
10–11	100	3	100	3	0
15.5–16.5	100	4	100	4	0
18.5–19.5	100	4	100	4	0
23–23.5	100	3	100	3	0
26.5–27.5	0	----	20	1	0
28.5–29.5	20	1	20	1	0
32–34	0	----	0	----	0

[a] 1 indicates the least rapid and 4 the most rapid decay.

The temperatures below 20° C. were maintained in an incubator where the humidity was quite high; but in the incubators at higher temperatures the humidity was correspondingly lower, and the oiled paper bags did not entirely prevent the desiccation of the decaying bulbs. Thus the possible influence of humidity as a limiting factor must be considered. It is to be seen that in this experiment the most rapid decay occurred at 15° to 20°, while above 26° the amount of infection and the rate of decay were decidedly limited.

Another series of experiments was run to determine whether infection would be accelerated at the higher temperatures if a high relative humidity prevailed. Two chambers in which the temperature was 25° to 27° and 28° to 30°, respectively, were kept at saturation. Inoculated bulbs were placed in open boxes in each. At the same time boxes of inoculated bulbs were placed in other chambers having different temperatures and different relative humidities. In the latter series the environment was not controlled as accurately as was desired, but the average temperatures and relative humidities as recorded on thermographs and hygrographs are given, and the results are included for their comparative value. (Table 4).

TABLE 4.—*The relation of temperature and humidity to decay of onion bulbs by Botrytis allii and B. byssoidea; 10 bulbs in each lot; Red Wethersfield variety*

Temperature °C.	Relative humidity per cent	Botrytis allii, per cent infected	Botrytis byssoidea, per cent infected	Control, per cent infected
10°±	60±	100	100	[a] 10
15°±	50–75	100	100	0
15°–20°	75–85	100	100	0
22°–25°	60–80	100	100	0
22°–27°	60–75	70	80	0
25°–30°	75–85	70	20	0
25°–27°	100	100	100	0
28°–30°	100	0	0	0

[a] One of 10 bulbs became infected by *Botrytis allii* during the experiment.

At temperatures below 20° C. the results were similar to those in the previous experiment. At temperatures above 20° the relative humidity was higher than in the previous experiment. Here it is evident that the higher humidity accelerated infection, although as was noted before, the progress of decay was distinctly retarded as the temperature rose above 22°. A comparison of the lots held at 100 per cent relative humidity at 25° to 27° and 28° to 30° shows that the maximum temperature for infection is somewhere between 27° and 30°. It is also significant that throughout these experiments *Botrytis allii* and *B. byssoidea* reacted very similarly to temperature. In Figure 1 are shown the relative amounts of decay in bulbs inoculated with *B. allii* and *B. byssoidea*, respectively, and held in moist chambers at 18°, 23°, and 26°.

Two experiments of a similar nature were run with *Botrytis squamosa*. The bulbs were placed in oiled paper bags in an incubator at temperatures ranging from 23° to 4° C. Infection occurred over the entire range. The most rapid decay took place at about 15°. At 18° it was nearly as rapid as at 15°. At 23° it was distinctly retarded, but desiccation was somewhat greater than at the lower temperatures. Below 15° there was a gradual slowing up of activity roughly proportionate to the reduction in temperature.

RECAPITULATION

The data which have just been noted point to the conclusion that all three fungi are favored by temperatures between 15° and 20° C. It is true that mycelial development is somewhat more rapid and

conidial germination a little prompter between 20° and 25°, but on the other hand the infection studies show quite clearly that the diseases progress less rapidly above 20° than between 15° and 20°. Sporulation in *Botrytis allii* and *B. byssoidea* occurs over a wide range if the atmosphere is humid, and this holds true even in the saprophytic stage of the fungi during the growing season of the host. *B. squamosa* is more limited in this respect, inasmuch as there appears to be little or no sporulation above 20°.

It may thus be assumed that humid cool weather during the growing season of the onion plant is most favorable to the development and spread of the neck-rot fungi, while hot dry weather is correspondingly less favorable. The small sclerotial neck-rot organism is probably the one most retarded by higher temperatures, and this belief is supported by the fact that it is usually not found in midwestern districts until some weeks after the appearance of mycelial neck rot. At harvest time, when infection of the bulbs commonly occurs, warm dry weather is least favorable to infection, and in fact incipient infection of mycelial neck rot has often been found to be completely checked by such an environment.

As substantiating these views some evidence from field observations on the occurrence of mycelial neck rot over a period of years in southeastern Wisconsin may be cited. In this district two severe epiphytotics have occurred in the decade from 1915 to 1924. In 1915 the disease was very severe even in the resistant Red Globe variety, while in 1924 the damage to this variety was nearly as great, and with the white varieties it approached a complete loss. In contrast with these two years, the season of 1919 was one of great scarcity of neck rot. Climatically, 1915 and 1924 represent one extreme and 1919 the other. An examination of the weather records taken by the United States Weather Bureau at Racine, Wis., give some tangible details. The season of 1915 was one of abundant rainfall, the total precipitation from June 1 to September 30, inclusive, being 15.76 inches, which was 2.92 inches above normal. The mean temperatures for June, July, and August were, respectively, 3.8°, 4.3°, and 4.4° F. below normal, while that for September was 1.4° above normal. In the season of 1924 the precipitation was 6.76 inches above normal, while the mean temperatures of the four months were, respectively, 2.2°, 0.7°, 1.3°, and 4.3° below normal. In 1919 the precipitation for the four-month period was only 0.17 inch above normal, while the mean temperatures were 5.9°, 4.9°, 1.4°, and 3.2° above normal. During the 1915 and 1924 periods there were, respectively, 77 and 49 days of cloudy or partly cloudy weather, while in 1919 there were only 37 such days. Thus the two epiphytotic years were unusually moist, cool, and cloudy during the major portion of the onion-growing season; while the year of least disease of the decade was unusually dry, warm, and clear during the corresponding period.

It is to be expected, from what we know of the life history of the Botrytis forms, that moist, cool, cloudy weather is most favorable to the saprophytic development of the organism during the growing season of the onion. The same conditions are noticeably effective in delaying the maturity of the crop and tend to develop plants

which are more succulent at the neck and thus slower to desiccate at the point where Botrytis infection occurs. Furthermore, such weather is most conducive to infection at the critical time. This fact was most noticeable in 1915 when maturity was delayed some three weeks, and the frequent rains during the harvest period made proper curing almost impossible. In 1924 the delay of harvest was not so great, but careful examination showed the organism to be widespread and fruiting abundantly on old leaves and the dying tips of the leaves just previous to pulling of the crop. The clear, hot, drying weather of 1919, on the other hand, was unfavorable to the multiplication and spread of Botrytis during the season, a fact which tended to reduce the amount of natural inoculum at harvest time. Moreover, the maturity of the host plant was hastened under such conditions, and the top growth was so reduced as to facilitate rapid and thorough desiccation of the neck. It seems quite likely that the influence of climatic conditions is exerted in three important directions: First, they create conditions favorable or unfavorable to the multiplication of the parasite; second, they prolong or shorten the period of growth of the host and influence the degree of succulency of the neck at harvest time; third, they influence infection at the critical time by either favoring or retarding the germination and growth of the causal organism.

RELATION OF STAGE OF MATURITY OF HOST TO INFECTION OF BULBS

It has already been intimated that the condition of the host tissues at the time of exposure to the parasites is important in determining infection. Some laboratory experiments will be reported which were designed to study this point.

LABORATORY EXPERIMENTS WITH BOTRYTIS ALLII

EXPERIMENT 1

Three varieties of onion, White Portugal, Yellow Globe, and Red Globe, were grown on soil which had not produced this crop for at least five years. When the tops of the plants had fully matured the bulbs were pulled. Two groups from this lot of bulbs were prepared. In one group tops were left intact; in the other they were clipped sufficiently near the bulb to expose the succulent tissues of the scales. The two groups were again divided into equal parts, one part in each case being used as a control, the other for inoculation. The controls were sprayed with sterile water, while the remaining bulbs were sprayed with a conidial suspension of *Botrytis allii*. The controls and the inoculated bulbs were then placed in separate wire baskets and kept in a room having a fairly constant temperature of about 18° C., where they were suspended in a partially closed chamber over moistened sphagnum moss. The inoculations were made on September 3, and the bulbs were examined on September 22. The final data are noted in Table 5.

TABLE 5.—*Development of Botrytis allii in topped and untopped bulbs of three varieties of onion; experiment 1*

Variety	Method of handling tops	Inoculated		Control	
		Total number of bulbs	Number with neck rot	Total number of bulbs	Number with neck rot
White Portugal	Topped	20	17	20	0
Do	Untopped	20	0	20	0
Yellow Globe	Topped	10	8	10	0
Do	Untopped	10	1	10	0
Red Globe	Topped	10	5	10	0
Do	Untopped	10	0	10	0

The outstanding result is that whereas infection took place quite readily when the succulent tissue was exposed, only 1 bulb out of the 40 which were exposed to the inoculum with the tops intact became diseased.

EXPERIMENT 2

The experiment reported above was repeated with some modifications. Red, Yellow, and White Globe plants were pulled, and the tops were allowed to cure out thoroughly. They were then divided into four groups and prepared for inoculation in the following ways: (1) The tops were cured down but not removed; (2) 1 to 2 inches of thoroughly cured neck were left intact; (3) no neck tissue was left, but since the tops were thoroughly cured down to the bulb proper no succulent tissue was exposed; (4) the necks were so removed as to expose wounded, succulent tissue. A portion of each lot was then sprayed with a conidial suspension of *Botrytis allii;* a second portion was sprayed with conidial suspension and covered with moistened filter paper; a third portion was sprayed with sterile water and covered with moistened filter paper to serve as a control. All three lots were stored in shallow trays at about 18° C. The inoculations were made on September 23. The final observations, made on November 27, are recorded in Table 6.

TABLE 6.—*Relation of neck tissue to infection of Botrytis allii in bulbs of White, Yellow, and Red Globe varieties; experiment 2*

Condition of neck tissue	Variety	Inoculated and not covered		Inoculated and covered with moistened filter paper		Sprayed with sterile water and covered with moistened filter paper	
		Number of bulbs	Number with neck rot	Number of bulbs	Number with neck rot	Number of bulbs	Number with neck rot
Untopped	White	25	0	27	1	26	0
	Yellow	25	0	27	0	25	0
	Red	25	0	26	0	25	0
1 to 2 inches of the neck left intact	White	25	0	25	0	25	0
	Yellow	49	0	27	0	3	0
	Red	29	0	26	0	28	0
No neck tissue left; succulent tissue not exposed	White	25	0	25	0	17	0
	Yellow	25	0	25	0	27	0
	Red	25	0	27	0	24	0
Tops clipped so as to expose wounded tissue	White	25	9	23	11	25	0
	Yellow	25	6	29	15	25	0
	Red	25	1	30	13	25	0

It is again evident that the desiccated neck tissue is, under the conditions of the experiment, an effective barrier against the invasion of *Botrytis allii*.

LABORATORY EXPERIMENT WITH BOTRYTIS BYSSOIDEA

EXPERIMENT 3

With bulbs from the same lot used in experiment 1 a comparable experiment was conducted at the same time, wherein inoculations were made by spraying with a conidial suspension of *Botrytis byssoidea*. Inoculation was made on September 3 and the final data were recorded on September 22. (Table 7.)

TABLE 7.—*Development of Botrytis byssoidea in topped and untopped bulbs of three varieties of onion; experiment 3*

Variety	Condition of tops	Inoculated bulbs		Control bulbs	
		Total number	Number with neck rot	Total number	Number with neck rot
White Portugal	Topped	21	19	20	0
Do	Untopped	19	3	20	0
Yellow Globe	Topped	10	8	10	0
Do	Untopped	10	1	10	0
Red Globe	Topped	10	8	10	0
Do	Untopped	10	0	10	0

It is evident here, as with *Botrytis allii*, that the removal of the top and exposure of the wounded succulent tissue favors infection. In this instance a few more bulbs with tops intact were infected than was the case with *B. allii*. The difference is not great enough, however, to warrant the conclusion that *B. byssoidea* is more aggressive as an invader through the uninjured neck tissue, although this question deserves further study.

It is to be expected that inception of the neck-rot diseases in the field may be influenced by the condition of the neck tissues of the bulb when it is exposed to the parasites under favorable environment. In the experiments just described the bulbs were not exposed to the organisms until the tops were entirely mature. In the light of this evidence it might be expected that bulbs which were thoroughly mature before being subjected to the organisms would remain reasonably free from infection. The opportunity for prompt and thorough desiccation of the neck tissues does not always prevail in the field. In general those conditions which favor sporulation of *Botrytis allii* and *B. byssoidea* and subsequent germination of conidia are the ones which inhibit rapid curing of the onion tops. It remained for actual field experimentation to determine whether or not the best natural curing of bulbs with the tops left intact was sufficient to ward off infection by the neck-rot fungi.

FIELD DATA

The field studies were carried on in the onion-growing section of eastern Racine County, Wis. An area was selected where the crop had been grown repeatedly for many years and where mycelial neck rot commonly occurs on the stored crop. Unfortunately for this

study, the gray-mold neck rot occurs only rarely in this section. The small sclerotial neck rot sometimes occurs, but it is confined to the white varieties, and they are grown to a very limited extent in this section. The field observations are therefore confined almost entirely to *Botrytis byssoidea*.

The first series of experiments was planned to compare the amounts of neck rot which developed in bulbs with mature and with immature necks at harvest. It was necessary to harvest such bulbs from practically the same spot and to handle them in the same manner in order to give equal exposure to natural inoculum and environing influences. Consequently a portion of a field was selected in which the crop was approaching maturity and in which the plants were ripening somewhat unevenly. The bulbs were harvested and divided into two groups designated as "mature" and "immature" according to the succulence of the neck tissues. They were then topped and placed in slatted crates, where they were allowed to cure in the open for some weeks, after which they were stored in an onion warehouse. The data obtained at the end of the storage periods are given in Table 8.

TABLE 8.—*Relation of maturity of neck tissue to natural infection of Red Globe onions by Botrytis byssoidea*

Year	Immature		Mature	
	Total number of bulbs	Percentage with neck rot	Total number of bulbs	Percentage with neck rot
1917	600	6.5	600	2.8
1918	363	21.2	661	8.6

Although the percentage of infected bulbs in the mature lots was small in both years, the amount was at least doubled in each case in the immature lots. These results are in line with what might be expected from the foregoing laboratory data.

The next matter of importance was to determine whether leaving the tops intact after harvest is a reliable barrier against infection. The possibility that it is, is suggested by the laboratory experiments, inasmuch as clipping the tops while only partially mature exposes the succulent wounds to natural inoculum. It is quite possible, however, that the fungus may become established in the dying tops before they are completely desiccated and thus gain ready access to the scales. Field experiments over a period of four years give a fair index as to the bearing of this point of natural infection. In each season from 1917 to 1920, inclusive, several bushels from the same area were harvested with and without tops. They were handled otherwise like the commercial crop. In 1917 and 1918 Red Globe variety was used. In 1919 White Globe and Red Globe varieties were used, but no neck rot developed in either lot; hence no report is included in the table. In 1920 Red, White, and Yellow Globe were used. In the last season two harvests were made. The first was on September 6, when the tops were not quite mature; the second was on October 9, some three weeks after harvest would normally occur, during which time they were exposed to some rainy weather. The percentage of neck-rotted bulbs was determined at the end of the storage period, and the data are recorded in Table 9.

TABLE 9.—*The effect of the removal of onion tops upon infection by Botrytis byssoidea; experiments at Racine, Wis., 1917–1920; data at end of the storage period*

Year and variety	Topped bulbs		Untopped bulbs	
	Total number	Percentage with neck rot	Total number	Percentage with neck rot
1917:				
Red Globe	1,228	5.1	1,164	0.6
1918:				
Red Globe	292	8.2	292	2.7
1920 (harvest on Sept. 6):				
Red Globe	243	0.0	194	0.0
Yellow Globe	224	0.0	214	0.0
White Globe	266	40.2	340	37.3
1920 (harvest of Oct. 9):				
Red Globe	340	0.2	365	0.6
Yellow Globe	323	5.5	297	0.0
White Globe	338	55.6	304	51.9

The data show the striking difference in susceptibility between colored and white varieties, a matter which will be discussed later. It is evident also that the bulbs in the earlier harvest, even though the tops were not quite mature at the time of harvesting, acquired less neck rot than did those which were harvested later, when the tops were mature. This is contradictory to the data previously discussed, in which mature and immature plants harvested at the same time showed the opposite results as to amount of infection. The bulbs harvested in early September had the advantage of good curing weather, which is also unfavorable for production of Botrytis conidia and infection. Those bulbs which remained longer in the field were exposed to environmental conditions more conducive to neck-rot infection. This is evidence therefore that the weather conditions are quite as important as the degree of maturity. Considering the effect of the removal of tops, it is evident that the bulbs of the white variety with tops left intact were affected nearly as severely as those which were topped. In the laboratory experiments (experiments 1, 2, and 3) the neck tissue of the bulbs was quite thoroughly desiccated before it was exposed to the organism. In the field this is not necessarily the case, and the difference in the field and laboratory results can only be explained by the supposition that infection in the field took place before the tops were completely cured. It is further evident that the harvesting of bulbs with the tops intact can not be relied upon as an effective means of control with the white varieties.

VARIETAL RESISTANCE

The fact has become well-established among onion growers and dealers that, of the varieties which are commonly grown for storage, the colored-bulb types have much better keeping qualities than the white-bulb type. The degree of susceptibility of the different varieties to the three neck-rot decays also tends to substantiate this fact. Munn (*20*) points out this difference in varietal susceptibility to *Botrytis allii*. In the Middle West it has been repeatedly observed in connection with *B. byssoidea*, and the results of a critical comparison are given in Table 9. Up to the present, *B. squamosa*

has been found in nature only upon white bulbs or (in one instance) on bulbs having a very slight yellowish color. The high resistance of yellow and red bulbs is shown by the fact that in several cases where the small sclerotial neck rot occurred on white onions, colored bulbs grown next to them were completely free from the disease.

There is abundant evidence, however, that colored bulbs are not resistant once infection is established. In nature they are not always completely free from the gray-mold or mycelial neck rots. The difference between the amount of disease in colored and white varieties is usually striking, but such colored bulbs as become infected decay quite as readily as the white bulbs. Repeated comparative inoculations have been made with each of the three neck-rot organisms upon red, yellow, and white bulbs. The results have uniformly shown that infection by way of wounds in the succulent scales is attained quite as readily in one variety as in another, while the progress of decay following infection is approximately the same in colored and white bulbs inoculated with any one of the three forms of neck rot. Resistance in the colored bulbs appears to be due to the exclusion of the fungi. The outer scales and the neck tissue of pigmented bulbs contain a water-soluble substance which is decidedly toxic to the Botrytis forms as well as to certain other onion-bulb pathogenes. It has been suggested that this toxin may prevent the invasion of the neck tissue by the Botrytis organisms and thus preclude infection. Further details of this phase of the investigation have been presented earlier (*32*, *35*).[3]

CONTROL

The control of the neck rots of onion has not been completely worked out as yet. The wider use of colored varieties has served to reduce the losses in a considerable measure. The greatest hazard is encountered when the white varieties are grown. There is little in the way of crop rotation and sanitation with an intensively produced crop like the onion which can be relied upon to reduce the disease in environments favorable to it. The most hopeful measure so far devised is that of artificial curing of the bulbs after harvest to check the organisms in severely infected lots. The investigations, which so far have dealt primarily with mycelial neck rot, show that rapid desiccation of the neck tissues, even though infection has already become evident, will to a great extent prevent further advance of the disease. Preliminary results have already been published (*33*), and further experimental work is under way.

SUMMARY

Three closely related but distinct neck-rot diseases of onion bulbs have been described. They are distinguished as follows: Gray-mold neck rot (*Botrytis allii* Munn), mycelial neck rot (*B. byssoidea* Walker), and small sclerotial neck rot (*B. squamosa* Walker).

The symptoms of each disease have been described and the morphology and pathogenicity of each organism discussed.

The three organisms are readily distinguished by the character of their growth upon potato-dextrose agar.

[3] In the papers cited *Botrytis byssoidea* is referred to as *Botrytis* sp. 110 and *B. squamosa* is referred to as *Botrytis* sp. 108a.

All three organisms will produce growth on potato-dextrose agar over a range of 3° to 33° C., with most profuse development from about 20° to 25°. Spore germination occurs over a range of 3° to 27° but most promptly from about 19° to 27°. *Botrytis allii* and *B. byssoidea* sporulate at temperatures from 4° to 25° or higher, but *B. squamosa* seldom produces spores at temperatures above 20°. Infection and decay of the bulbs, however, are clearly favored most by temperatures below 20° and above 15°.

Field observations in the Middle West over a period of years show that *B. byssoidea* at least has been most prevalent during seasons in which the temperature was, on an average, below normal and the rainfall above normal.

Infection by *Botrytis allii* and *B. byssoidea* takes place more readily when the neck tissue is succulent at the time of exposure than when it has become desiccated. When harvested under comparable conditions, bulbs with immature tops showed a higher percentage of infection by *B. byssoidea* than did bulbs with mature tops. The removal of tops at harvest predisposes the bulbs somewhat more to infection by *B. byssoidea*. The difference between results with topping the bulbs and harvesting them with tops intact is not great enough, however, to warrant recommendation of the latter procedure as a means of control.

Colored varieties are in general less subject to attack by these three neck-rot organisms than are white varieties. When infection once occurs, however, decay proceeds with equal rapidity in both types. It is suggested that the water-soluble toxin present in the dry outer scales and neck tissue of colored bulbs aids materially in excluding the organisms.

The disease may be controlled in a large measure by the use of colored varieties. Artificial curing of the bulbs after harvesting so as rapidly to desiccate the neck tissues is also effective in checking the disease.

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FUSARIA CAUSING BULB ROT OF ONIONS[1]

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INTRODUCTION

Immediately upon institution of cooperative work in 1917 between the fruit and vegetable inspection service of the Bureau of Agricultural Economics and the Bureau of Plant Industry, it was found that a bulb rot is an important factor in the market and transit losses of onions. Fusarium species were found associated with this rot. Reports of the disease in the field had been made by Selby (*8*)[2] from Ohio in 1910, by Hanzawa (*5*) from Japan in 1914, and by Clinton (*3*, *4*) from Connecticut in 1915.

In the fall of 1920, the attention of the senior writer was called to serious field losses in the onion fields near Grand Junction and Delta, in the Uncompaghre Valley of Colorado. It was found also that during the previous winter exceedingly heavy losses had occurred during storage of onions in this section. The disease appeared to be like the bulb rot which had been noted repeatedly on the market in imported and domestic onions from various parts of the United States (*6*). Material was collected in the Colorado fields, and this became the basis for intensive study of the disease.

During 1921 the bulb rot was a serious factor in onion shipments from Texas. Some rot was also noted on the market in shipments from Colorado. During 1922 material was collected in the fields near Stockton, Calif., Walla Walla, Wash., and again in the Uncompaghre Valley of Colorado. It was found at Walla Walla that the bulb rot had been becoming an increasingly serious factor in the production and marketing of onions (*2*). In this section the onion crop matures in the summer. The disease not only cuts the crop short by causing premature death of the tops and small-sized bulbs but also necessitates heavy culling and immediate marketing under refrigeration to reduce losses from development and spread of the rot after harvest. Much material was collected in Walla Walla by the senior writer, and later more was sent from there by G. R. Isaman and Carl Dysart.

After the writers had begun work on the onion bulb-rot problem, it developed that J. C. Walker (*12*) was carrying on studies of a bulb rot of onions which occurred in the onion-set producing sections near Chicago. It was agreed that he should continue his studies of the Illinois bulb rot and that the writers would pursue their studies of the material which they had collected.

The results reported in this paper are based on studies of material collected in the markets of the United States, especially in Chicago since 1917, and in the field, especially in Colorado and Washington.

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[2] Reference is made by number (italic) to "Literature cited," p. 951.

Two hundred isolations have been made from affected bulbs. Single-spore cultures have been made from one-half of these. The distribution of the sources of these cultures is as follows: California (Stockton), 10; Colorado (Delta), 33; Illinois, 6; Egypt, 2; Spain, 4; Texas, 6; and Washington (Walla Walla), 131. To begin with, single-spore cultures were made of all material as it was received, and these were used for cultural and inoculation experiments. It was found impossible to continue the work on this scale, and as a result the collections made since 1923, with a few exceptions, have not been grown as single-spore cultures, and cultural and inoculation studies have been limited to about 100 single-spore cultures.

The cultures derived from single spores were grouped as much as possible on the basis of macroscopic characters. One group of 26 organisms and a few individual cultures from other groups were used for intensive work. It was found that even this one group of apparently similar organisms contained more than one species.

The situation is comparable to the potato Fusarium problem, and much work remains to be done. The major difficulty lies in the taxonomy of the form genus Fusarium, (*1*) which is fully appreciated only by those who have done more than desultory work with these fungi. Clearly, it is not advisable to defer publication until all the organisms collected are studied and identified.

This paper reports the results of an intensive study of the cultural characters and pathogenicity of 10 organisms whose final classification was made at the Fusarium conference, with the help of H. W. Wollenweber (*17*) and C. D. Sherbakoff.[3] It is hoped that this work can be continued so as to clear up the onion Fusarium situation.

THE DISEASE

On the market and in storage, bulb rot of onions has been found in different degrees of severity in onions from practically all large onion-growing sections of the United States. It occurs also in onions from Egypt and Spain. It has been found that there is a definite correlation between abundance and severity of the disease in transit and storage, and in the field. This was clearly brought out by the Walla Walla situation. The fact that Fusarium species are soil inhabitants occurring all over the world makes it probable that Fusarium bulb rot will be found wherever temperature conditions are favorable for infection after onion culture has been carried on long enough to give rise to an abundant source of inoculum in the soil.

Walker and Tims (*12, p. 683–684*) give the following description of the disease as it occurs in the field in the Chicago region and in the greenhouse:

> A progressive yellowing and dying back from the tips of the leaves is the first sign. The rapidity of this development will vary, sometimes the aerial part dying completely within one or two weeks and in other cases the decay extending over a much longer period. Appearance of newly affected plants may continue until harvest. When the early signs of the disease appear above ground, decay has already started at the stem plate. (Fig. 1, A.) The roots commonly turn pink and gradually decay, until eventually the entire root system may disappear. A semiwatery decay affecting all the tissues of the succulent scales starts from the base upward. (Fig. 1, A, B, C.) In early infections this decay may continue so as to almost completely destroy the bulb by harvest. (Fig. 1, B.) In

[3] The Fusarium conference was held at Madison, Wis., in 1924. The writers are indebted to the following who took part in it: H. W. Wollenweber, C. D. Sherbakoff, O. A. Reinking, and Helen Johann.

FIG. 1.—Fusarium bulb rot of young onion plants collected in the field at Walla Walla, Wash., June, 1923: A, withered and yellowed tops, the first field symptom; A, B, and C, dead roots (note progress of the lesion from the scale plate); B, bulb almost completely decayed, a white hyphal mass showing between the scales

other cases incipient infections at harvest continue to advance during storage and transit, finally leaving dry, shrivelled mummies. In the case of red onions it is common for the anthocyan pigment in the outer epidermis to turn green in color for a considerable distance in advance of the actual decay, indicating a change in reaction of the cell sap. Fusarium infection of the bulb is often associated with insect wounds, and since this organism is primarily a wound parasite the coincidence of severe epiphytotics of the disease and maggot injury is common.

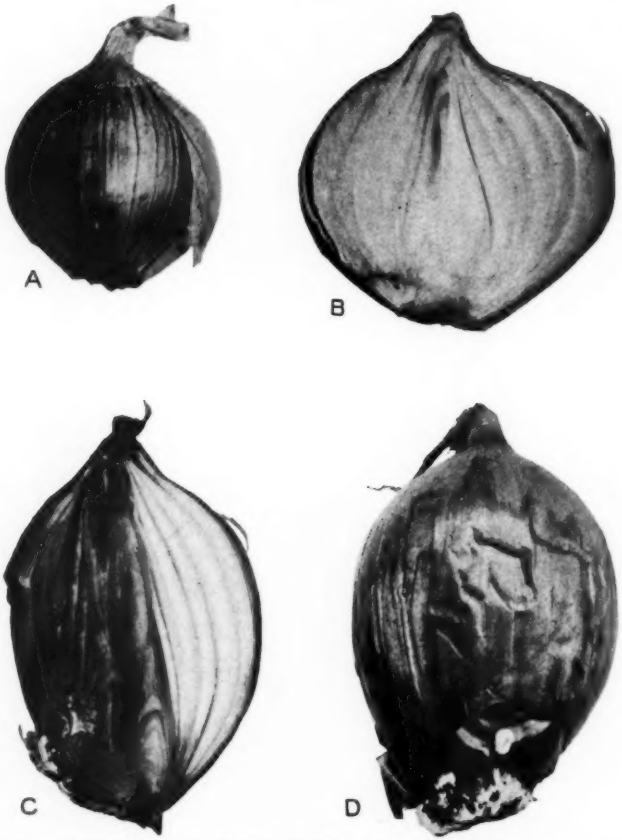

FIG. 2.—Fusarium bulb rot following inoculation with a pure culture of *F. zonatum* forma 1. A and B, controls showing wound made by inoculation; C, a section through D, cut so that the wound is visible at the surface with a mass of hyphae. A mealy decay extends from the wound, involving the scale plate and half of the bulb up to the neck. The decayed half is collapsed; D, exterior of an onion showing shriveling and superficial growth of the mycelium at the scale plate as well as at the opening of the wound

Observations of plants in the field made by the writers toward the close of the growing season in California, Colorado, and Washington, and of plants grown and inoculated experimentally in the greenhouse, indicate that this description is generally applicable.

The symptoms of affected bulbs vary with the moisture content of the bulb and of the air. So far there seems to be no difference in symptoms correlated with different species of Fusarium, as in the

tuber rots of the Irish potato. Freshly infected bulbs show nearly vinaceous buff to avellaneous [4] mealy decay extending upward from the scale plate, which in a humid atmosphere usually becomes covered with a white to pinkish cottony mycelium. (Fig. 2, D.) If the bulb at this stage is exposed to dry air, it loses water rapidly, the scales become tough and leathery, and the bulb becomes a mummy. However, if the bulb is kept under moist conditions the scales become filled with mycelium, which forms rows of cushions extending along the veins, and these are visible through the epidermis. (Fig. 2, C.) If the bulb reaches this stage before drying out, the affected scales, when they do dry out, are powdery and full of spores. There is no foul odor in bulbs affected with Fusarium bulb rot. When the bulbs are kept in moist air bacteria often become active, and in conjunction with Fusarium species, cause a semiwatery to watery decay which often is foul smelling. This decay, due to bacteria and Fusarium species, is not, however, as wet or slimy as that caused by bacteria alone.

It should be pointed out that Fusarium species, which upon inoculation into a bulb do not produce a decay by themselves, when associated with bacteria progress through the scales of the bulb, producing the plectenchymatic cushions and spore masses present in bulbs attacked by truly pathogenic Fusaria. The decay so produced differs from true Fusarium decay only in that it is semiwatery instead of mealy and has a foul odor, but can not be distinguished from that caused by a pathogenic species plus bacteria. This accounts for the fact that many bulbs naturally infected yield only nonpathogenic Fusaria when they are cultured.

PROOF OF PATHOGENICITY

To demonstrate the pathogenicity of the organisms isolated from decaying onions, inoculation experiments were conducted with growing plants and with mature bulbs.

Inoculation of Sets

Sets of red, yellow, and white onions were selected for freedom from disease. They were placed for 15 minutes in 1:1,000 corrosive sublimate solution and then rinsed in sterile distilled water. They were planted in soil which had been autoclaved at 15 pounds for two hours. Eight-inch pots were used and five bulbs were planted in each. Two of the bulbs of each pot were wounded by cutting a small piece out of the side of the scale plates (1 mm. wide, 4 to 5 mm. long, and 3 mm. deep). The bulbs were placed on pieces of Melilotus stems inoculated with the culture under test.

There was no stunting or evidence of disease until the plants were approaching maturity. (Fig. 3.) Plants grown in inoculated soil did not mature faster than the controls. The leaves of both controls and plants in inoculated soil had begun to dry back from the tips and some plants had set seed before the first symptoms of disease developed. (Fig. 4, B.) At this stage plants in the inoculated pots collapsed suddenly with yellowing and wilting. This occurred during a period of exceedingly hot weather following a sudden rise of temperature (fig. 5), and is in harmony with the findings of Walker and Tims (*12, p. 693*). Examination of the bulbs of such collapsed

[4] Designations of color are made according to Ridgway (7).

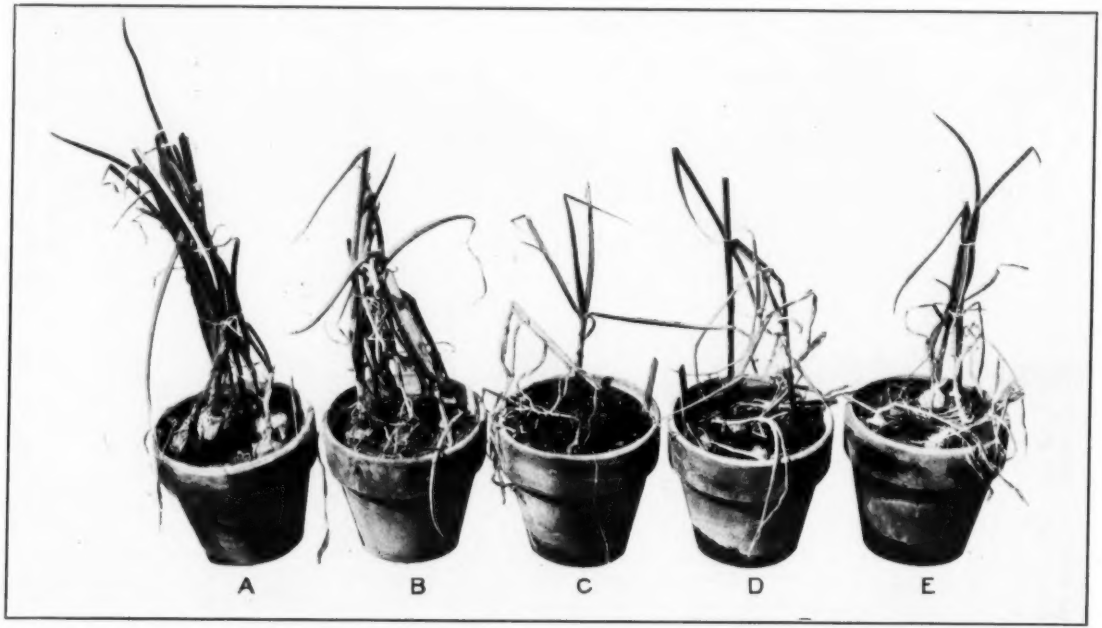

FIG. 3.—Soil-infection experiment conducted in the greenhouse with various species of Fusarium. Age of plants, 15 weeks. A, inoculated with a strain of *F. moniliforme* (1624–b′); B, control; C, inoculated with *F. zonatum* forma 1 (1490); D, with *F. zonatum* forma 2 (1456–c); and E, with *F. cepae* Hanz. emend. (1440–c). All bulbs in A and B were sound and free from Fusarium species. Symptoms of disease did not become apparent in the other plants until they had begun to mature. All the bulbs were examined and cultured. The bulbs of dead and dying plants in C, D, and E were decayed, and the inoculated Fusarium species were recovered from them in culture

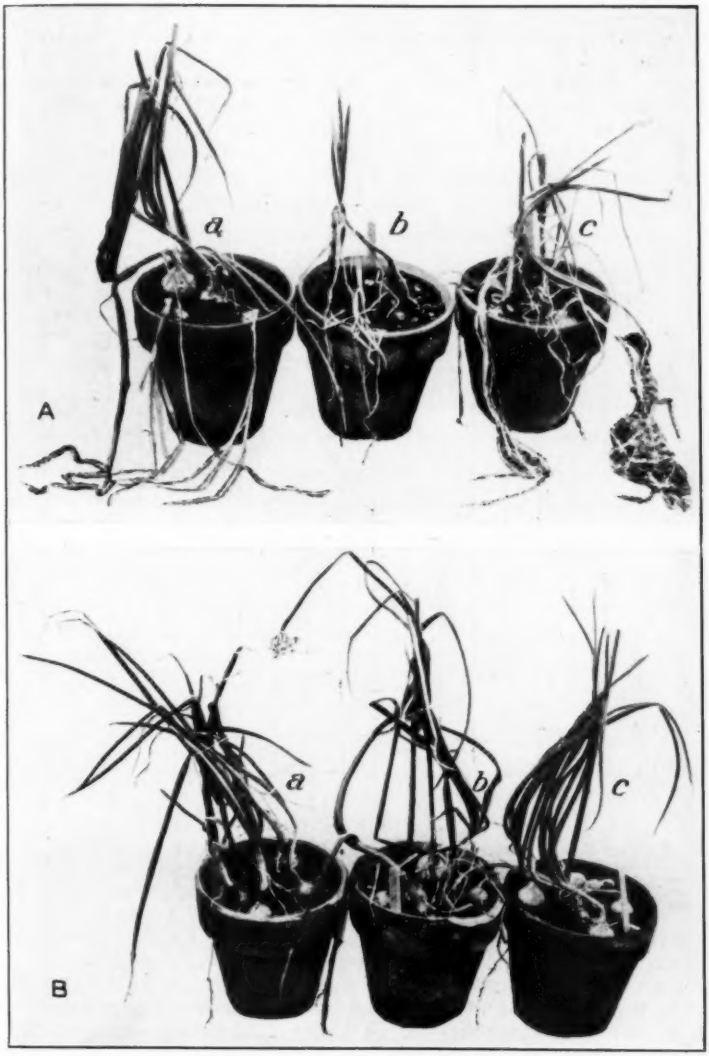

FIG. 4.—Soil inoculation experiments with red, white, and yellow varieties of onions. A, soil inoculated with *Fusarium zonatum* forma 1: *a*, white variety; *b*, red; *c*, yellow. B, soil inoculated with *F. malli*: *a*, red variety; *b*, yellow; *c*, white. Age of plants in A and B, 15 weeks. Death of tops in A was due to age and bulb decay; in B to age alone

plants revealed typical bulb rot and the inoculated organisms were recovered by culture. (Table 1.) In some cases the rot was associated with maggot injury in the nonwounded bulbs. The bulbs of the controls were examined critically to determine whether the dying-back noted in the leaves could be associated with any abnormal condition of the bulbs. Out of 45 controls only one bulb showed decay. It had been injured by maggots and mites, and bacteria and a Fusarium belonging to the section Martiella, none of which were found to be pathogenic to onions, developed in culture.

Fusarium malli (fig. 4, B), *F. vasinfectum*, *F. oxysporum*, *F. martii* var. *minus*, *F. bulbigenum*, and *F. moniliforme* (fig. 3, A), all isolated from decayed bulbs, were used in this experiment, but did not produce

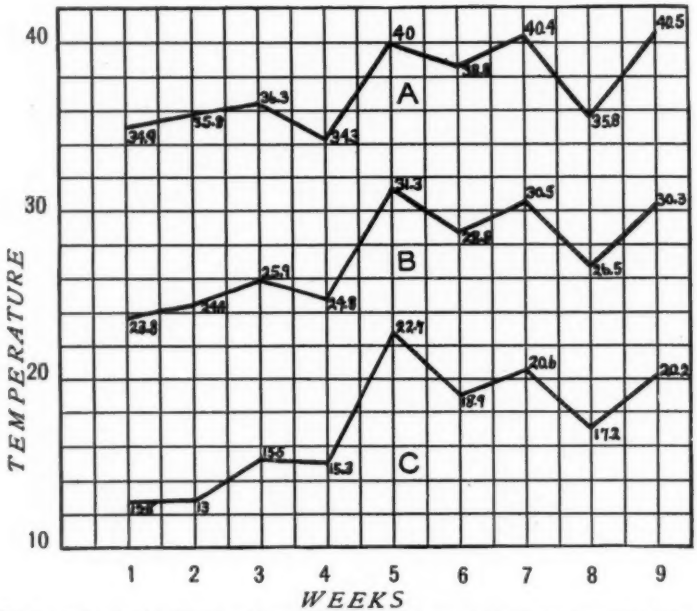

FIG. 5.—Graph showing average maximum, mean, and minimum air temperatures prevailing during the last nine weeks (May 5 to June 30) of soil inoculation experiment begun March 19, 1925. The first symptoms of the disease did not appear until June 12, immediately following the onset of high temperatures. A, maximum (average); B, mean (average); C, minimum (average). Temperature in degrees centigrade

decay. In bulb inoculations these also proved nonpathogenic with the exception of *F. moniliforme*, which sometimes produced rot.

Some of the onions from inoculated soil, which showed no bulb rot at the conclusion of the experiment in the greenhouse, were held to determine whether they would develop bulb rot in storage. They were stored in clean paper bags at room temperature (20 to 24° C.) for several months. At the end of this period some of these bulbs had developed typical bulb rot. (See Table 2.) The lesions were cultured and the inoculated organism was recovered. These experimental facts show that bulbs, apparently free from decay and wounds, harvested from infested soil can develop the disease in transit and storage.

TABLE 1.—*Inoculation of sets*

Culture	Red				Yellow				White			
	Wounded		Unwounded		Wounded		Unwounded		Wounded		Unwounded	
	Number of bulbs tested	Number of bulbs decayed	Number of bulbs tested	Number of bulbs decayed	Number of bulbs tested	Number of bulbs decayed	Number of bulbs tested	Number of bulbs decayed	Number of bulbs tested	Number of bulbs decayed	Number of bulbs tested	Number of bulbs decayed
1456-c (F. zonatum forma 2)	4	1	6	2	4	1	6	[a] 3	4	1	6	0
1490 (F. zonatum forma 1)	4	4	6	0	4	2	6	3	4	1	6	1
1440-c (F. cepae)	4	1	6	0	4	[b] 2	6	1	4	2	6	1
1660 (F. malli)	4	0	6	0	4	0	6	0	4	0	6	0
1324 (F. vasinfectum)	4	0	6	0	4	0	6	0	4	0	6	0
1626-b (F. oxysporum)	4	0	6	0	4	0	6	0	4	0	6	0
1495 (F. martii var. minus)	4	0	6	0	4	[c] 2	6	0	4	0	6	[c] 1
1624-b (F. moniliforme)	4	0	6	0	4	0	6	0	4	[d] 1	6	0
1407 (section Martiella)	4	0	6	0	4	0	6	0	4	0	6	0
1455-d (F. bulbigenum)	4	0	6	[a] 1	4	[c] 1 / [a] 1	6	0	4	0	6	0
Control	6	0	9	0	6	0	9	0	6	[f] 1	9	0

[a] 1 bacteria and maggots.
[b] 1 plus maggots.
[c] Bulb injured by sow bugs; rot plus bacteria.
[d] Slight.
[e] Rot plus Penicillium.
[f] Bacteria and Martiella group Fusarium plus maggots and mites.

TABLE 2.—*Greenhouse onions kept in storage*

Culture No.	Onion variety	Wounded or unwounded	Number of bulbs stored	Number of bulbs showing decay
1624-b′ (F. moniliforme)	White	Wounded	2	0
	Yellow	do	2	0
1660 (F. malli)	Yellow	Unwounded	1	0
	White	do	1	0
1455-d (F. bulbigenum)	Yellow	Unwounded	2	0
	Red	Wounded	1	0
	White	do	1	0
	do	Unwounded	2	0
1626-b (F. oxysporum)	Yellow	Wounded	2	0
	do	Unwounded	3	0
	Red	Wounded	1	0
	do	Unwounded	1	0
	Yellow	Unwounded	1	0
	do	Wounded	1	[a]
1407 (section Martiella)	White	do	1	0
	do	Unwounded	1	0
	Red	Wounded	1	0
	do	Unwounded	1	0
	Red	Wounded	1	[a]
1456-c (F. zonatum forma 2)	Yellow	Unwounded	1	0
	White	do	2	1
1495 (F. martii minus)	White	Unwounded	1	[b]
	Red	do	1	1
1490 (F. zonatum forma 1)	Yellow	Wounded	1	1
	White	do	1	1
1440-c (F. cepae)	Red	Unwounded	2	1
	White	do	1	1

[a] Rot plus Penicillium. [b] Penicillium only.

BULB INOCULATIONS

For these experiments disease-free bulbs were selected of white, yellow, and red varieties. The bulbs were washed, immersed in 1:1,000 mercuric chloride for one hour and then rinsed in sterile

water. Early tests with wounded and nonwounded bulbs indicated that the Fusarium species pathogenic to onion bulbs are wound parasites. (See Table 3.) For this reason in all later pathogenicity tests, a stab was made with a sterile probe just above and extending into the scale plate. (Fig. 2, A, B.) The inoculum consisted of spores taken from 10-day-old potato dextrose tubes and was inserted in the stab with a needle. The bulbs were then placed in sterile moist chambers and incubated at 25° C. for 48 hours. They were next put into clean new paper bags (a bag for each species of Fusarium) and stored in a cabinet at room temperature (20 to 24° C.) for periods of two weeks to four months. Adequate controls were run.

TABLE 3.—*Summary of inoculation of wounded and unwounded bulbs*

[Single-spore cultures were used for inoculations]

Culture No.	Locality from which onion was obtained	Region from which isolation was made	Inoculations	
			Wounded	Unwounded
299	Spain	Bulb	+	−
404	Texas	Bulb (side lesion)	−	−
454–a	Colorado	Bulb	−	−
454–b	do	do	+	−
455	do	do	+	−
456	do	do	+	−
486	do	do	−	−
487	do	do	+	−
488	do	do	+	−
490	do	do	+	−
491–a	do	do	−	−
491–b	do	do	−	−
524–a	do	do	+	Rhizopus.
524–b	do	do	Penicillium	−
524–c	do	do	+	−
524–d	do	do	+	−
592	Spain	do	−	−
592–a	do	Same bulb as 592	−	−
592–b (a)	do	Bulb	−	−
592–b (b)	do	Same bulb	+	−
602	Illinois	Onion set	−	−
673	Colorado	Bulb	−	Rhizopus.
840–a	Texas	do	+	−
840–b	do	do	+	−
1182	Spain	do	+	−
1260–b	Colorado	do	+	−
1263	do	do	+	−
1264	do	do	−	−
1322	do	do	+	−
1323	do	do	+	−
1324	do	do	−	−

Generally mycelium developed in the mouth of the wounds in 48 hours, even in those onions which did not become diseased. In those which rotted a depressed area developed about the mouth of the wound in 4 to 5 days, depending upon the temperature. The sunken area increased but not commensurately with the volume of the affected tissues. (Fig. 2, D.) Affected tissues appeared plump, but when pressure was applied were found to be spongy instead of firm.

There were very few contaminations and these were due to bacteria and to *Penicillium* spp. (Table 4.) The latter did not markedly affect the nature of the decay but bacteria did, changing the decay from a mealy to a watery one.

TABLE 4.—*Summary of inoculation experiments of wounded bulbs*

[Inoculations made with single-spore cultures]

Culture No.	Locality from which bulb was obtained	Region of bulb from which isolation was made	Group to which culture belongs	Number of inoculations	Number of takes
1490	D[a]	B[b]	Elegans (zonatum forma 1)	28	28.
1491	D	B	do	14	14.
1459–b	W	B	do	15	15.
1451–c	W	(B)	do	16	16.
1430–a	W	B	do	14	14.
1440–c	W	(B)	Elegans (cepae emend.)	28	25.
1456–c	W	(B)	Elegans (zonatum forma 2)	28	25.
1458–a	W	B	do	14	14.
1443–b	W	B	do	14	11.
1475–d	W	B	do	14	14.
1454–b	W	B	do	16	16.
1484–a	W	(B)	Elegans	17	14.
1455–d	W	B	Elegans (bulbigenum)	30	4, slight; 6, rot plus bact.[c]
1426–B	W	B	Elegans	14	0.
1450	D	B	do	15	0.
1496–x	D	B	do	14	0.
1493	D	B	do	14	3?
1440–ax	W	(B)	do	14	3; 1, rot plus bact.
1433–b	W	(B)	do	14	2, rot plus bact.
1475–b	W	B	do	14	0.
1471–b	W	(B)	do	14	1.
1429–c	W	(B)	do	14	0.
1457–d	W	(B)	do	14	0.
1446–e	W	B	do	14	0.
1409	S	B	do	10	8.
1322	D	B	Liseola (F. moniliforme)	12	10.
840–b	T	B	do	10	2; 2, rot plus bact.
1624–b′	W	Stem plate	do	16	3, plus bact; 4, rot plus bact.
1324	D	Side lesion of bulb	Elegans (F. vasinfectum)	19	2, slight.
1495	D	B	Martiella (F. martii minus)	15	0.
1407	S	R	Martiella	16	1, slight; 1, rot plus bact.
1626–b	W	B	Elegans (F. oxysporum)	14	0.
1475–c	W	R	Elegans	14	5, slight; 5, rot plus bact.
486	D	B	Martiella	12	1, rot plus bact; 1, rot plus Penicillium.
1264	D	B	Discolor	11	5, slight.
592	Spain	B	Elegans	10	1, slight; 4, rot plus bact.
299	Spain	B	do	6	3, slight.
1397	S	Stem plate	do	6	0.
1401	S	R	do	6	0.
1404–a	S	R	do	6	0.
1404–B	S	R	do	6	0.
1405	S	R	Martiella	6	0.
1406–a	S	R	do	6	2 (1 slight).
1406–B	S	R	Elegans	6	5.
1426–A	W	B	do	6	6.
1427–A	W	(B)	do	6	6.
1427–B	W	(B)	do	6	5; 1, Penicillium.
1429–A	W	(B)	do	6	6.
1430–c	W	(B)	do	6	5.
1431–d	W	(R)	do	6	5; 1 Penicillium.
1432–a	W	B	do	6	0.
1432–c	W	R	do	6	6.
1432–E	W	Bulb of 1432–c	do	6	2; 1 rot plus bact.
1433–d	W	B	do	6	5; 1 contaminated.
1434–B	W	(B)	do	6	6.
1434–d	W	(B)	do	6	6.
1435–b	W	Stem plate	do	6	3.
1435–d	W	(B)	do	6	5; 1 contaminated.
1436–a	W	B	do	6	0.
1436–c	W	Root of 1436–a	Martiella	6	5.
1437–b	W	B	Elegans	6	5.
1437–d	W	B	do	6	6.
1438–a	W	B	do	6	6.
1438–c	W	B	do	6	2, slight; 1, rot plus bact.
1439–b	W	B	do		

[a] D=Delta, Colo.; S=Stockton, Calif.; T=Texas; W=Walla Walla, Wash.

[b] B=bulb with roots intact; (B)=bulb with roots entirely destroyed by decay; R=root from rotted bulb; (R)=pink roots from bulbs without bulb rot.

[c] "Plus bact."=bacteria present in addition to Fusarium.

TABLE 4.—*Summary of inoculation experiments of wounded bulbs*—Continued

Culture No.	Locality from which bulb was obtained	Region of bulb from which isolation was made	Group to which culture belongs	Number of inoculations	Number of takes
1439–c	W	Stem plate	Elegans	6	6.
1440–a	W	(B)	do	6	6.
1441–a	W	(R)	Martiella	6	0.
1442–a	W	(B)	do	6	2, slight.
1442–c	W	(B)	Elegans	6	6.
1443–d	W	B	do	6	6.
1445–b	W	(B)	do	6	5.
1445–d	W	(B)	do	6	5; 1, contaminated.
1446–b	W	(B)	do	6	6.
1447–a	D	(B)	do	6	5.
1447–c	D	(B)	Martiella	6	0.
1448–a	D	(B)	Elegans	6	6.
1449–c	D	(B)	do	6	4.
1451–b	W	(B)	do	6	6.
1452–b	W	B	do	6	6.
1452–c	W	B	do	6	4; 2, contaminated.
1456–a	W	(B)	Martiella	6	1, rot plus bact.
1457–a	W	B	Elegans	6	5.
1457–ax	W	(B)	do	6	6.
1458–d	W	B	do	6	4.
1460–d	W	(B)	do	6	5.
1461–a	W	B	do	6	5.
1472–a	W	B	do	6	4.
1472–ax	W	B	do	6	6.
1473–a	W	(B)	do	6	4.
1473–d	W	(B)	do	6	6.
1474–b	W	B	do	6	4.
1475–a	W	R	do	6	5.
1488	D	B	do	6	6.
1492	D	B	do	8	2; 2, rot plus bact
1494	D	B	do	6	2; 2, rot plus bact
1494–x	D	B	do	6	6.
1496–a	D	B	Martiella	6	3 (2 slights).
1496–b	D	B	Elegans	6	4 (2 con.).

It should be noted that with but one exception (1475–a, which belongs to section Elegans) strains isolated from the roots did not produce bulb rot. Additional work with other cultures not listed in the table indicates that, with few exceptions, the organisms occurring on roots (*10*) are not pathogenic to bulbs. It is significant in this connection that inoculation of bulbs with *F. malli*, Taub., which is reported to be the cause of pink root (*11*), proved negative (Tables 1 and 6.)

VARIETAL SUSCEPTIBILITY

No difference in susceptibility in red, white, and yellow onion varieties was noted, as indicated by Tables 1 and 5 and Figure 4.

TABLE 5.—*Summary of inoculations of different varieties of onions*

Culture	Red		Yellow		White	
	Number tested	Number decayed	Number tested	Number decayed	Number tested	Number decayed
1496–a (section Martiella)	1	0	1	0	1	0
1404–a (section Elegans)	1	0	1	0	1	0
1490 (*F. zonatum* forma 1)	3	(a)	2	2	2	2
1456–c (*F. zonatum* forma 2)	3	3	2	2	2	2
1626–b (*F. oxysporum*)	3	0	2	0	2	0
1440–c (*F. cepae* emend)	3	2	2	2	2	(b)
1455–d (*F. bulbigenum*)	3	0	2	0	2	(c)
1495 (*F. martii* var. *minus*)	3	0	2	0	2	0
1624–b′ (*F. moniliforme*)			2	0	2	0
1324 (*F. vasinfectum*)			2	0	2	0
1407 (section Martiella)			2	0	2	0
Control	2	0	3	0	2	0

[a] 3 (1 slight). [b] 2 (1 slight). [c] 1? (in dead tissue).

IDENTITY OF THE PATHOGENES

A study of cultures shows that with the exception of 840–b, 1624–b′, and 1322, which belong to section Liseola, all of the pathogenic forms fall into section Elegans (*13*). It also shows, however, that by no means all species of Elegans isolated from onions are pathogenic.

In their early grouping the writers found a considerable number of organisms which developed an abundant pionnotes. Of these cultures 1490 and 1491 from Delta, Colo., and 1459–b, 1451–c, and 1430–a from Walla Walla, Wash., were found to be identical. Culture 1490 was selected for study in determining this species. It was identified by the writers as *F. zonatum* (Sherb.) Wollenweber (*15*). It was also compared with a culture furnished by Walker and Tims which they had described as *F. cepae* (Hanzawa) emend. Walker and Tims (*12*). The writers' culture 1490 seemed identical not only with *F. zonatum* but also with *F. cepae* furnished them by Walker and Tims. At the Fusarium conference it was decided that the organism described by Walker and Tims as *F. cepae* emend. Walker et Tims (*12*) is *F. zonatum* forma 1 and that 1490 is identical with it.

The description of 1490 follows.

STRAIN 1490—FUSARIUM ZONATUM (SHERB.) WR. FORMA 1

Mycelium typically scant, when present fine fluffy white to grayish white.

Substratum typically light buff to pale ochraceous buff, rarely on very acid media in dim light from vinaceous fawn to russet vinaceous (the deep colors described by Walker and Tims (*12, p. 684*) for their strain were never obtained with 1490); no sclerotia but small warm sepia to warm blackish brown plectenchymatic cushions on steamed rice; color on rice from cream white to salmon buff or salmon color; slight lilac odor on rice; chlamydospores (fig 6, B) abundant in spores and mycelium, terminal and intercalary, solitary, in chains, and in clusters, smooth to echinate; microconidia (fig. 6, A) usually not numerous, scattered in aerial mycelium or present in sporodochia or pionnotes, ellipsoidal to ovoid or slightly dosiventral, mostly 0-septate 7.8 by 2.7μ average (5 to 11.6 by 1.6 to 4.15μ), some 1-septate 13.2 by 2.7μ average (8 to 18.2 by 1.6 to 3.32μ); macrospores (fig. 6, A and B) sickle-shaped, pedicellate, slightly constricted at apex, borne on irregularly branched sporophores, scattered or in sporodochia and pionnotes; pionnotes typically present, salmon buff to ochraceous salmon; macroconidia 3 to 5-septate, predominately 3-septate 37.1 by 3.8μ average (27.2 to 46.5 by 2.8 to 4.56μ); 4-septate 41.5 by 3.92μ average (34.4 to 50 by 3.2 to 4.56μ); 5-separate 43.4 by 4μ average (37 to 49.8 by 3.32 to 4.56μ).

Zonation marked on some media.

Habitat: On decaying bulbs of onion (*Allium cepa* L.), Delta, Colorado.

Measurements [5] of spores on different media [6] are as follows (numbers in parentheses after averages indicate number of spores measured. At least five fields were counted for percentages):

On potato-dextrose-agar [7] tube, culture 35 days old, conidia from pionnotes:
5-septate, 8 per cent, 41.8 by 4.2μ average (5), 37 to 45 by 4 to 4.5μ.
4-septate, 38 per cent, 41.4 by 4.1μ average (10), 39 to 43 by 4 to 4.5μ.
3-septate, 54 per cent, 38.7 by 4.1μ average (10), 37 to 41 by 4 to 4.5μ.
On oatmeal-agar tube, culture 26 days old, conidia from pionnotes:
5-septate, 2.8 per cent, 45.5 by 4μ average (5), 43 to 48 by 3.32 to 4.15μ.
4-septate, 6.7 per cent, 43.5 by 4.1μ average (10), 38.2 to 50 by 3.32 to 4.5μ
3-septate, 49.6 per cent, 41.5 by 4μ average (15), 34 to 45 by 3.32 to 4.5μ.
2-septate, 2.5 per cent.
1-septate, 1.5 per cent, 14.3 by 2.9μ average (3), 10 to 18.2 by 2.5 to 3.32μ.
0-septate, 36.9 per cent, 8.8 by 2.7μ average (10), 5 to 11.6 by 1.7 to 3.32μ.

[5] Only normal spores were measured. For criteria of the norm see (*9, 14, 17*).
[6] The formulae followed for making media are those used at the Fusarium conference (*17*) or those given by Sherbakoff (*9*).
[7] All potato-dextrose agar, unless otherwise stated, is 2 per cent dextrose and 2 per cent agar.

On oatmeal-agar plate, culture 36 days old, conidia from pionnotes:
5-septate, 1.5 per cent.
4-septate, 15.4 per cent, 38.4 by 3.4μ average (5), 34.4 to 40 by 3.2 to 3.6μ.
3-septate, 80.2 per cent, 36 by 3.4μ, average (11), 27.2 to 43.2 by 2.8 to 3.6μ.
0-septate, 2.9 per cent.
Chlamydospores, 6.5 by 6.7μ average (4), 5.6 to 8 by 4.8 to 8μ.
On potato-tuber plug, culture 18 days old, conidia from sporodochium:
5-septate, 0.5 per cent, 38.3 by 3.7μ average (4), 37 to 38.8 by 3.7μ.
4-septate, 3.7 per cent, 36.5 by 3.7μ average (8), 35 to 38.8 by 3.7μ
3-septate, 87.5 per cent, 33.3 by 3.6μ average (22), 27.8 to 40.7 by 1.9 to 3.7μ.
2-septate, 0.3 per cent.
1-septate, 6.7 per cent.
0-septate, 1.3 per cent.

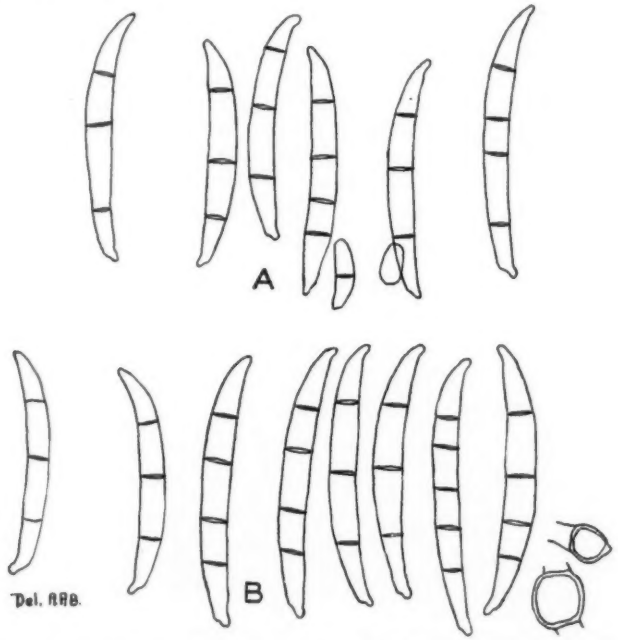

FIG. 6.—Spores of 1490, *Fusarium zonatum* (Sherb.) Wr. forma 1: A, spores from sporodochium on 35-day-old rice culture; B, macrospores and chlamydospores from pionnotes on 35-day-old potato-agar tube culture. Camera lucida drawings. All × 1,000

On potato-tuber plug, culture 37 days old, conidia from sporodochium:
5-septate, 1.2 per cent, 45 by 3.7μ average (2), 44 to 46.5 by 3.32 to 4.15μ.
4-septate, 23 per cent, 42.7 by 3.9μ average (10), 36.5 to 46.5 by 3.32 to 4.15μ.
3-septate, 73.2 per cent, 36.5 by 4μ average (20), 30 to 46.5 by 3.32 to 4.15μ.
1-septate, 1.2 per cent, 12.9 by 2.9μ average (7), 10 to 15.8 by 2.5 to 3.32μ.
0-septate, 1.4 per cent, 7 by 2.9μ average (10), 5 to 8.3 by 2.5 to 4.15μ.
On Melilotus stems, culture 26 days old, conidia from pionnotes:
4-septate, 1.5 per cent, 38.4 by 3.8μ average (2), 36.8 to 40 by 3.6 to 4μ.
3-septate, 64.5 per cent, 32.5 by 3.5μ average (10), 29 to 36 by 3.2 to 4μ.
1-septate, 1.5 per cent, 12.8 by 2μ average (4), 8 to 16 by 1.6 to 2μ.
0-septate, 32.5 per cent, 8.5 by 2.1μ average (3), 8 to 9.6 by 1.6 to 2.8μ.

On onion-agar tubes, culture 21 days old, conidia from pionnotes:
6-septate, 0.6 per cent.
5-septate, 1.5 per cent, 45.5 by 4.2μ average (7), 41.5 to 49.8 by 4.15 to 4.56μ.
4-septate, 10.6 per cent, 43.5 by 4μ average (15), 40 to 48 by 3.32 to 4.56μ.
3-septate, 34 per cent, 41.6 by 4μ average (20), 38.2 to 46.5 by 3.32 to 4.5μ.
Small 3-septate, 34 per cent, 27.5 by 3.5μ average (7), 25 to 31.5 by 2.9 to 4.15μ.
2-septate, 0.2 per cent, 16.6 by 3.32μ average (1).
1-septate, 2.4 per cent, 13.3 by 2.9μ average (5), 11.6 to 15 by 2.5 to 3.32μ.
0-septate, 50.7 per cent, 7.6 by 2.8μ average (11), 5 to 10 by 1.7 to 4.15μ.
On steamed rice, culture 35 days old, conidia from old sporodochium:
5-septate, 1 per cent.
4-septate, 12 per cent, 40.5 by 4μ average (2), 38 to 43 by 4μ.
3-septate, 67 per cent, 35.6 by 3.82μ average (10), 33 to 38 by 3.5 to 4μ (30 by 3.5μ).
2-septate, 1 per cent.
1-septate, 7 per cent.
0-septate, 12 per cent.
On 20 per cent dextrose-agar plate, culture 40 days old, conidia from pionnotes:
4-septate, 2.9 per cent.
3-septate, 12.8 per cent.
2-septate, 3.1 per cent.
1-septate, 0.7 per cent.
0-septate, 80.5 per cent.
Average of all media:
5-septate, 43.4 by 4μ average (23), 37 to 49.8μ by 3.32 to 4.56μ.
4-septate, 41.5 by 3.92μ average (62), 34.4 to 50 by 3.2 to 4.56μ.
3-septate, 37.1 by 3.8μ average (118), 27.2 to 46.5 by 2.8 to 4.56μ.
Small 3-septate, 27.5 by 3.5μ average (7), 25 to 31.5 by 2.9 to 4.15μ.
2-septate, 16.6 by 3.32μ average (1).
1-septate, 13.2 by 2.7μ average (19), 8 to 18.2 by 1.6 to 3.32μ.
0-septate, 7.8 by 2.7μ average (34), 5 to 11.6 by 1.6 to 4.15μ.
Chlamydospores, 6.5 by 6.7μ average (4), 5.6 to 8 by 4.8 to 8μ.

One culture of the pionnotes group, 1440–c (from Walla Walla), which is pathogenic, differed from all the others. It seemed to be close to *Fusarium sclerotioides* Sherb. and *F. aurantiacum* (Link, Sacc.). The description of *F. sclerotioides* more fully fits the organism because of the reference to hyphal knots and wartlike plectenchyma bodies (*9, p. 215*). At the conference mentioned above it was agreed that *F. sclerotiodes* was *F. aurantiacum*, but that the writers' 1440–c differed so much from *F. aurantiacum* that it should be considered a different species. In view of the fact that the name *F. cepae* had been used and the meager description given by Hanzawa (*5*) was not at variance with that of 1440–c, except for the greater diameter of spores (4.5 to 6.3μ given by Hanzawa), it was decided that 1440–c should be described as *F. cepae* Hanzawa emend. Hanzawa's figures show abnormal, swollen conidia, and it is likely that he used these for his measurements. Wollenweber in his discussion of Fusarium in Sorauer's "Handbuch" (*16, p. 173*) has called attention to this possibility.

The morphological and cultural characters of the organism have been determined on various media and are given below.

Fusarium Cepae Hanz. Emended

Fine, fluffy white aerial mycelium, sometimes Botrytis green at base of slant or on agar rich in dextrose; Artemisia green plectenchyma on potato-tuber plugs; substratum usually colorless but on agar very rich in dextrose (20 per cent) dark vinaceous purple; color on steamed rice salmon buff to light salmon orange; sclerotia on potato-tuber plugs and agar, one-half millimeter in diam-

eter, dark dull yellowgreen to duck green; loose hyphal knots to irregular plectenchymatic bodies 1 to 2 millimeters in diameter, white to pale salmon buff, sometimes present in plate cultures. These may bear sporodochia; Chlamydospores (fig. 7, A, *c*, *d*, *e*, and E, *b*) abundant in mycelium and conidia, terminal and intercalary, unicellular (6.4 by 6.4μ) to two celled, solitary, in chains, or in groups, smooth to echinate; microconidia (fig. 7, A *a*, B, E, *a*), borne scattered and in false heads on the aerial mycelium, also present in sporodochia and pionnotes, ellipsoidal to ovoid or slightly dorsiventral, mostly unicellular, 6 to 9 (13) by 2 to 3.25μ (7.5 by 2.7μ average), rarely 1-septate, 11.5 to 17 (20) by 2 to 3μ (12.7 by 2.75μ average); Aurantiacumlike macroconidia (fig. 7, A, C, D, E) borne on irregularly branched conidiophores, scattered or in sporodochia or pionnotes, light vinaceous cinnamon to ochraceous salmon, sickle-shaped, pedicellate, slightly constricted at apex, 3 to 5 septate (rarely 2 to 6), predominately 3-septate 25 to 46 by 3 to 4.5μ (35.7 by 3.8μ average), on some media producing in addition small 3-septate spores, 22.5 to 28 by 2 to 3μ (25.7 by 2.7μ average); 2-septate 16.5 to 17.6 by 2 to 3μ (17.4 by 2.8μ average); 4-septate, 32 to 51 by 3 to 5μ (40.9 by 3.7μ average); 5-septate, 38 to 53 by 2.7 to 5μ (44.4 by 3.9μ average); 6-septate, 46.8 to 55 by 2.5 to 3.7μ (52.4 by 3.3μ average).

Differs from *F. aurantiacum* by the abundance of the chlamydospores in mycelium and conidia, by absence of red color on rice, and presence of slight lilac odor on steamed rice.

Habitat: On decaying bulbs of onion (*Allium cepa* L.) Walla Walla, Washington.

LATIN DESCRIPTION

Aerio mycelio subtile, ex albo, interdum "Botrytis green" ad radicem tubi agaris vel in *Solani tuberosi* agare perglucoso; stromate plectenchymico "Artemisia green" in tuberibus *Solani tuberosi;* substrato plerumque hyalino, sed "dark vinaceous purple" in *Solani tuberosi* perglucoso (20%); colore in Oryza cocta "salmon buff" vel "light salmon orange"; sclerotiis in tuberibus et agare *Solani tuberosi* (1–2 mm. diam.) "dark dull yellow green" vel "duck green"; laxis nodis in hyphalibus apicibus vel enormibus plectenchymis corporibus (1–2 mm. diam.), albido vel "pale salmon buff," interdum in culturis in Petri patina, interdum sporodochia ferentibus; chlamydosporis (fig. 7, A, *c*, *d*, *e*, and E, *b*) largis in mycelio et conidiis, terminalibus vel intercalaribus, continuis (6.4×6.4μ), 1-septatis, singulis, catenulatis, acervalibus, levibus vel rugulosis; microconidiis (fig. 7, A, *a*, B, E, *a*), aerio mycelio instratis vel capitulis falsis dispositis, quoque in sporodochiis vel in pionnote, ellipsoideo-ovoideis vel vix dorsiventralibus, plerumque continuis 6 to 9 (13) by 2 to 3.25μ (7.5 by 2.7μ average); rarius 1-septatis, 11.5 to 17 (20) by 2 to 3μ (12.7 to 2.75μ average); macroconidiis (fig. 7, A, C, D, E) similibus F. aurantiaco ad conidiophoros irregulariter ramosos dispositis, aerio mycelio instratis, vel in sporodochiis et in pionnote, "light vinaceous cinnamon" vel "ochraceous salmon," falcatis, pedicellatis, apice leviter constricto, 3 to 5 septate (raro 2 to 6 septate); plerumque 3 septate 25 to 46 by 3 to 4.5μ (35.7 by 3.8μ average); in aliquis mediis quoque minoribus 3 septate conidiis 22.5 to 28 by 2 to 3μ (25.7 by 2.7μ average); 2 septate 16.5 to 17.6 by 2 to 3μ (17.4 by 2.8μ average); 4 septate 32 to 51 by 3 to 5μ (40.9 by 3.7μ average); 5 septate 38 to 53 by 2.7 to 5μ (44.4 by 3.9μ average); 6 septate, 46.8 to 55 by 2.5 to 3.7μ (52.4 by 3.3μ average).

A *F. aurantiaco* (Lk.) Sacc. emend. Wr. differt abundantiis chlamydosporiis in mycelio et conidiis, nullo carmineo colore in Oryza cocta, et praesentia parvi odoris Syringae in Oryza cocta.

Habitat: In bulbis putridis *Allii cepae* L., Walla Walla, Washington.

Measurements of spores on different media are as follows:

On potato-dextrose agar tube, culture 31 days old, conidia from sporodochium:
- 3-septate, 74 per cent, 33.3 by 4μ average (10), 31 to 38 by 3.5 to 4.5μ (28 by 4).
- 1-septate, 1 per cent, 17 by 2.5μ average (1).
- 0-septate, 25 per cent.

On potato-dextrose agar tube, culture 75 days old, conidia from pionnotes:
- 5-septate, 2 per cent.
- 4-septate, 12 per cent.
- 3-septate, 57 per cent.
- 1-septate, 3 per cent.
- 0-septate, 26 per cent.

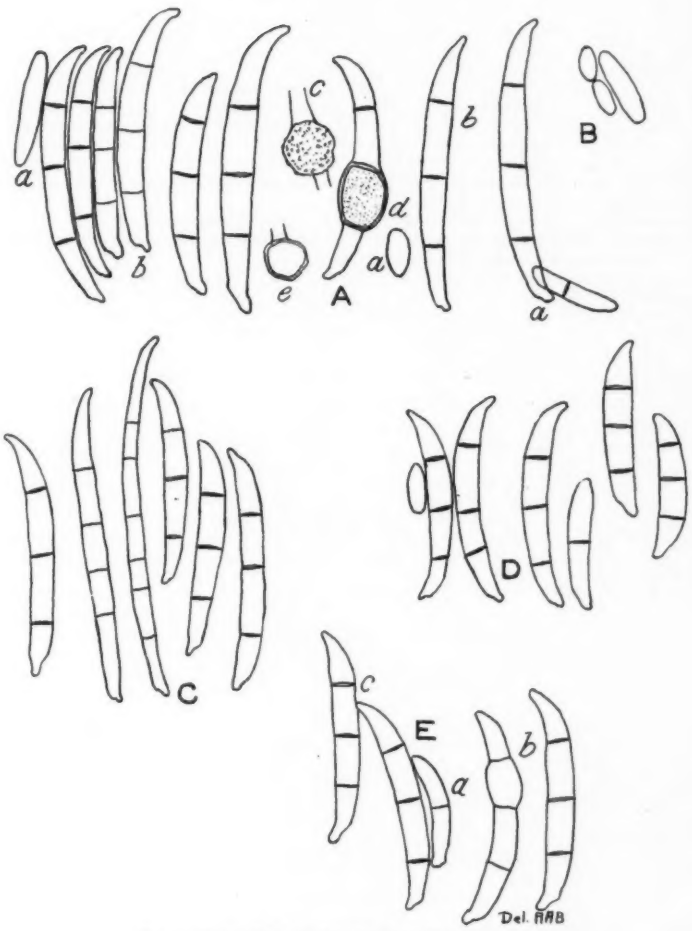

FIG. 7.—Spores of 1440-c, *Fusarium cepae* Hanzawa emended

A.—From pionnotes on oatmeal-agar culture 5 days old: *a*, microspores; *b*, macrospores; *c*, echinate intercalary chlamydospore from mycelium; *d*, chlamydospore in conidium; *e*, smooth terminal chlamydospore from mycelium

B.—Microconidia from 32-day-old steamed rice culture

C.—Types of macroconidia from thin submerged pionnotes on 32-day-old potato-tuber plug culture

D.—Conidia from sporodochium (1 mm. diameter) above the mycelium on a 32-day-old Melilotus stem culture

E.—Conidia from sporodochium on potato-agar tube culture 31 days old: *a*, microspore; *b*, chlamydospore in macrospore; *c*, macrospores

Camera lucida drawings. All × 1,000

On oatmeal-agar tube, culture 26 days old, conidia from sporodochium:
5-septate, 8 per cent, 47.3 by 3.93μ average (6), 46.5 to 49.8 by 3.32 to 4.15μ.
4-septate, 33 per cent, 43.3 by 3.93μ average (10), 39.8 to 51.5 by 3.32 to 4.15μ.
3-septate, 48 per cent, 40.5 by 3.98μ average (10), 36.5 to 43.2 by 3.32 to 4.15μ.
2-septate, 1 per cent, 17.6 by 3.15μ average (3), 16.6 to 18.3 by 2.9 to 3.32μ.
1-septate, 0.5 per cent, 11.5 by 3.00μ average (5), 10 to 13.3 by 2.5 to 3.32μ.
0-septate, 9.5 per cent, 7.5 by 2.65μ average (10), 5 to 11.6 by 1.66 to 3.32μ.

On oatmeal-agar plate, culture 5 days old, conidia from pionnotes:
5-septate, 6 per cent.
4-septate, 19 per cent.
3-septate, 74 per cent, 38 by 3.75μ average (10), 32.5 to 44 by 3 to 4.5μ.
1-septate, 1 per cent, 14 by 2.75μ (1).
0-septate, 13.5 by 3.25μ average (2), 9 to 18 by 3 to 3.5μ.

On potato-tuber plug, culture 15 days old, conidia from sporodochium:
7-septate, very rare, 46.8 by 3.9μ (1).
6-septate, very rare, 53.7 by 3.7μ average (2), 51.8 to 55 by 3.7μ.
5-septate, 15 per cent, 41.7 by 2.72μ average (8), 38.8 to 46.8 by 3.7 to 3.9μ.
4-septate, 24 per cent, 40 by 3.8μ average (13), 33.3 to 46.3 by 3.3 to 3.7μ.
3-septate, 59 per cent, 35.8 by 3.8μ average (33), 31.2 to 42.9 by 3.7μ.
0-septate, 2 per cent.

On potato-tuber plug, culture 32 days old, conidia from thin submerged pionnotes:
6-septate, 2 per cent, 54 by 2.75μ average (2), 53 to 55 by 2.5 to 3μ.
5-septate, 6 per cent, 47.5 by 3.25μ average (2), 47 to 48 by 3 to 3.5μ.
4-septate, 10 per cent, 41 by 3.75μ average (2), 40 to 42 by 3.5 to 4μ.
3-septate, 79 per cent, 33.2 by 3.8μ average (10), 28 to 37 by 3.5 to 4.5μ.
1-septate, 1 per cent.
0-septate, 2 per cent.

On potato-tuber plug, culture 37 days old, conidia from sporodochium:
5-septate, 10 per cent, 40 by 4.2μ average (10), 38.2 to 53.1 by 4.15 to 4.56μ.
4-septate, 32 per cent, 38.8 by 4.1μ average (10), 34.9 to 43.2 by 3.32 to 4.15μ.
3-septate, 47 per cent, 36.2 by 3.98μ average (10), 32.2 to 38.2 by 3.32 to 4.15μ.
1-septate, 3 per cent, 11.5 by 2.9μ average (5), 8.3 to 14.9 by 2.5 to 3.32μ.
0-septate, 8 per cent, 7 by 2.7μ average (10), 4.2 to 10.8 by 2.5 to 3.32μ.

On Melilotus stems, culture 26 days old, conidia from pionnotes:
Thin pionnotes on drier upper part of the tube: 4-septate, 5 per cent; 3-septate, 83 per cent; 0-septate, 12 per cent.
Thick pionnotes at moist bottom of tube: 4-septate, 1 per cent; 3-septate, 13 per cent; 1-septate, 2 per cent; 0-septate, 84 per cent.
Small 3-septate, 32 by 2.7μ average (10), 22.4 to 35.2 by 2.4 to 3.6μ.
2-septate, 17.6 by 2μ average (2), 17.6 by 2 to 2.4μ.
1-septate, 12.8 by 2μ average (3), 11.2 to 14.4 by 2 to 2.4μ.
0-septate, 9.6 by 2μ average (2), 7.2 to 12.8 by 1.6 to 2μ.
Chlamydospores 6.4 by 6.4μ average (3).

On Melilotus stems, culture 32 days old, conidia from 1 millimeter sporodochium above the mycelium:
3-septate, 24 per cent, 27.9 by 4.1μ average (10), 25 to 32 by 3.5 to 4.5μ (22 by 4).
2-septate, rare.
1-septate, 9 per cent, 20 by 3μ (1).
0-septate, 67 per cent, 7.5 by 2.5μ (1).

On onion-agar tubes, culture 27 days old, conidia from sporodochium:
5-septate, 2 per cent, 49.8 by 4.15μ average (10), 44.8 to 53.1 by 3.32 to 4.98μ.
4-septate, 17 per cent, 44.3 by 4.1μ average (10), 38.2 to 47.3 by 3.32 to 4.98μ.
3-septate, 68 per cent, 41.1 by 3.8μ average (10), 33.2 to 46.4 by 3.32 to 4.15μ.
Small 3-septate, 25.7 by 2.7μ average (10), 23.2 to 28.2 by 2.5 to 2.9μ.
2-septate, rare, 16.6 by 3.32μ (1).
1-septate, 2 per cent, 12.8 by 2.7μ average (10), 8.3 to 19.9 by 1.66 to 3.32μ.
0-septate, 11 per cent, 6.5 by 2.8μ average (10), 5 to 10 by 2.5 to 3.32μ.

On cornmeal agar, culture 9 days old, conidia from very thin pionnotes:
Macroconidia 99 per cent.
3-separate in majority.
4-septate and 5-septate common.
0 and 1 septate 1 per cent.
Average of all media:
6-septate, 52.4 by 3.3μ average (5), 46.8 to 55 by 2.5 to 3.7μ.
5-septate, 44.4 by 3.9μ average (39), 38 to 53 by 2.7 to 5μ.
4-septate, 40.9 by 3.7μ average (55), 32 to 51 by 3 to 5μ.
3-septate, 35.7 by 3.8μ average (110), 25 to 46 by 3 to 4.5μ.
Small 3-septate, 25.7 by 2.7μ average (10), 22.5 to 28 by 2 to 3μ.
2-septate, 17.4 by 2.8μ average (6), 16.5 to 17.6 by 2 to 3μ.
1-septate, 12.7 by 2.75μ average (26), 11.5 to 17 (20) by 2 to 3μ.
0-septate, 7.5 by 2.7μ average (35), 6 to 9 (13) by 2 to 3.25μ.

Some of the other organisms of the original pionnotes group proved identical. These were 1456–c, 1458–a, 1443–b, 1475–d, and 1454–b, all from onions from Walla Walla, Wash. Culture 1456–c was used in identification and was found to be identical with 1490, *F. zonatum* forma 1, in every respect except that in place of the salmon to salmon buff it constantly developed a lavender to red color on certain media. It is described as *F. zonatum* (Sherb.) Wr. forma 2 (nova forma).

The morphological and cultural characters of the organism were determined by cultures on various media and are as follows:

F. ZONATUM (SHERB.) WR. FORMA 2, NOVA FORMA

Fine, fluffy white to purplish lilac mycelium, typically scant on most media; substratum on oatmeal agar plates dull Indian purple; no sclerotia but small (1/2 mm.) warm sepia to warm blackish brown plectenchymatic cushions present on steamed rice, these sometimes giving rise to spore masses; color on rice Acajou red to Indian lake with light vinaceous lilac to carmine mycelium at top; slight lilac odor on rice; chlamydospores (fig. 8, C, *b*) abundant in spores and mycelium, terminal and intercalary, unicellular to two celled, solitary, in chains or in groups, smooth to echinate; microconidia (fig. 8, E) scattered in aerial mycelium or present in sporodochia or pionnotes, 0-septate mostly 7.9 by 2.8μ average (5 to 11.6 by 1.7 to 4.56μ), some 1-septate 14 by 3μ average (8.3 to 18.3 by 1.7 to 4.56μ); macrospores (fig. 8, A–E) borne on irregularly branched sporophores (fig. 8, D) in sporodochia and pionnotes, vinaceous cinnamon to buff pink or light ochraceous salmon, sickle-shaped, pedicellate, slightly constricted at apex, 3- to 6-septate, predominately 3-septate, 38.5 by 3.7μ average (24 to 48 by 2.4 to 4.56μ, on potato-tuber plugs an occasional small 3-septate spore (fig. 8, A, *a*) 19 by 2.8μ average (16.6 to 25 by 2.5 to 3.32μ); 2-septate rare, 18.1 by 3.2μ average (15 to 21.6 by 2.9 to 4.15μ); 4-septate 39.9 by 3.8μ average (31.5 to 53 by 3.2 to 4.56μ); 5-septate, 42.1 by 4.1μ average (36.5 to 50 by 3.2 to 5μ). Zonation sometimes marked on agar plates.

Differs from *Fusarium zonatum* forma 1 by the constant red color on rice and the lilac mycelium and vinaceous cast present in spore masses.

LATIN DESCRIPTION

Aerio mycelio subtile, ex albo vel "purplish lilac," plerumque exiguo; sporodochiis et pionnote; substrato in Avenae coctae agare "dull Indian purple," in acida agare *Solani tuberosi* "light perilla purple"; sclerotiis nullis, sed parvis ½ millimeter "warm sepia" vel "warm blackish brown" plectenchymicis pulvinis in oryza cocta, interdum sporodochia ferentibus; colore in oryza cocta "Acajou red" vel "Indian lake" cum "light vinaceous lilac" vel "carmine" mycelio in apice; parvo odore Syringae in oryza cocta; chlamydosporiis (fig. 8, C *b*) largis inconidiis et mycelio, terminalibus aut intercalaribus, continuis, 1–septatis, singulis, catenulatis, aut acervalibus, levibus vel rugulosis; microconidiis (fig. 8, E) aerio mycelio instratis, aut in sporodochiis vel in pionnote dispositis, ellipsoideo-ovoideis vel vix dorsiventralibus; plerumque continuis, 7.9 by 2.8μ average (5 to 11.6 by 1.7 to 4.56μ); 1-septate paucis 14 by 3μ average (8.3 to 18.3 by 1.7 to 4.56μ); macroconidiis (fig. 8 A–E) ad conidiophoros (fig. 8, D) irregulariter ramosos dispositis, liberis, in sporodochiis et in pionnote, "vinaceous

cinnamon," "buff pink," aut "light ochraceous salmon," falcatis, pedicellatis, apice leviter constricto; 3 to 6 septate; plerumque 3-septate 38.5 by 3.7μ average (24 to 48 by 2.4 to 4.56μ); in tuberibus Solani tuberosi aliquando minoribus 3-septate conidiis (fig. 8, A, *a*) 19 by 2.8μ average (16.6 to 25 by 2.5 to 3.32μ);

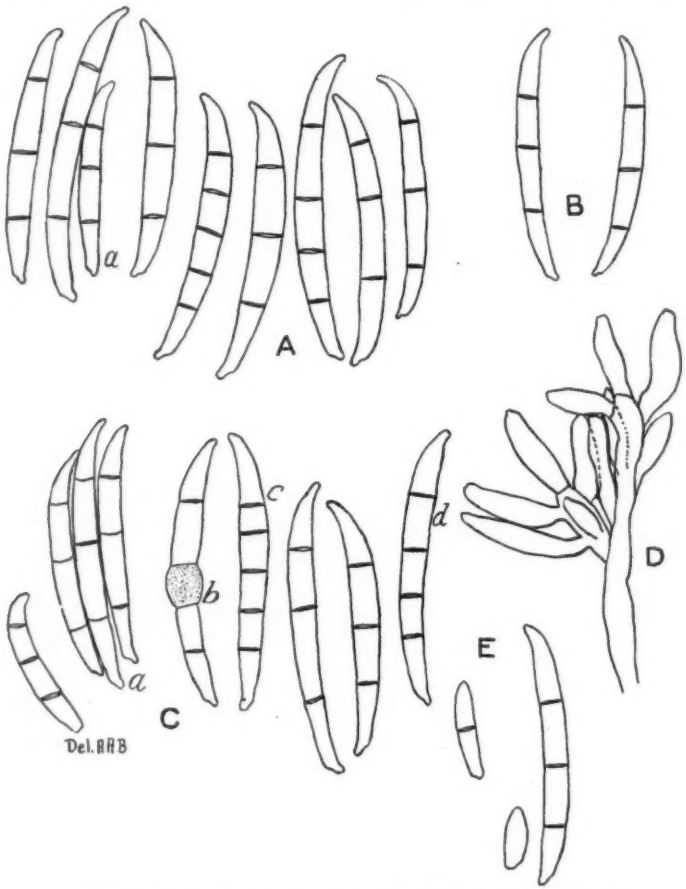

FIG. 8.—Spores of 1456-c, *Fusarium zonatum* (Sherb.) Wr. forma 2, nova forma

A.—Macrospores from dry sporodochium on top of 37-day-old steamed rice culture; *a*, small 3-septate spore
B.—Macrospores from pionnotes on 35-day-old potato-tuber plug culture
C.—Macrospores from pionnotes on 35-day-old potato-agar tube culture: *a*, 3-septate spores; *b*, Chlamydospore in macroconidium; *c*, 5-septate spore; *d*, 4-septate spore
D.—Sporophore from pionnotes on 13-day-old Melilotus stem culture
E.—Microspores and macrospore from pionnotes on 13-day-old Melilotus stem culture
Camera lucida drawings. All × 1,000

rarô 2-septate, 18.1 by 3.2μ average (15 to 21.6 by 2.9 to 4.15μ); 4-septate 39.9 by 3.8μ average (31.5 to 53 by 3.2 to 4.56μ); 5 septate, 42.1 by 4.1μ average (36.5 to 50 by 3.2 to 5μ); zonae interdum apertae in agare.

A. *F. zonato* forma differt constante rubro colore in *Oryza cocta*, ferme purpureo substrato, "lilac" mycelio, et "vinaceous" colore in conidiorum molibus.

Measurements of spores on different media are as follows:

On potato-dextrose-agar tube, culture 35 days old, conidia from pionnotes:
5-septate, 8 per cent, 42.5 by 4.3μ average (5), 41 to 43 by 4 to 4.5μ.
4-septate, 37 per cent, 41 by 4μ average (10), 38 to 43 by 3.75 to 4.5μ (23 by 3, 45 by 4μ).
3-septate, 53 per cent, 40.1 by 4μ average (10), 36 to 43 by 3.75 to 4μ (48 by 4).
1-septate, 1 per cent.
0-septate, 1 per cent.

On potato-dextrose-agar tube, culture 90 days old, conidia from pionnotes:
6-septate, 2.7 per cent.
5-septate, 18.7 per cent.
4-septate, 27.0 per cent.
3-septate, 47.9 per cent.
0-septate, 3.7 per cent.

On oatmeal-agar tube, culture 26 days old, conidia from pionnotes:
4-septate, 4.7 per cent, 46.3 by 4.15μ average (10), 41.5 to 53 by 3.73 to 4.56μ.
3-septate, 89.9 per cent, 43 by 3.7μ average (10), 40 to 45.6 by 3.32 to 4.15μ.
2-septate, rare, 21.6 by 4.15μ (1).
1-septate, 1.8 per cent, 20 by 3.32μ (1).
0-septate, 3.6 per cent, 8 by 2.7μ average (5), 5 to 11.6 by 2.5 to 3.32μ.

On oatmeal-agar plate, culture 36 days old, conidia from pionnotes:
5-septate, 5.4 per cent, 40 by 3.4μ average (2), 40 by 3.2 to 3.6μ.
4-septate, 18.4 per cent, 37.8 by 3.2μ average (3), 36.8 to 38.4 by 3.2μ.
3-septate, 61.6 per cent, 33.6 by 2.9μ average (10), 32 to 36.8 by 2.4 to 3.6μ.
0-septate, 14.6 per cent.

On potato-tuber plug, culture 18 days old, conidia from sporodochium:
6-septate, 0.4 per cent.
5-septate, 0.4 per cent, 39 by 3.7μ average (2), 37 to 40.7 by 3.7μ.
4-septate, 0.4 per cent, 37.3 by 3.73μ average (9), 33.3 to 42.5 by 3.7 to 4.16μ.
3-septate, 93 per cent, 34 by 3.7μ average (15), 29.6 to 40.7 by 3.7 to 4.16μ.
2-septate, 0.4 per cent.
1-septate, 1.4 per cent.
0-septate, 4.0 per cent.

On potato-tuber plug, culture 35 days old, conidia from pionnotes:
4-septate, 6 per cent, 42 by 4μ (1).
3-septate, 94 per cent, 37.3 by 3.5μ average (3), 36 to 39 by 3.5μ.

On potato-tuber plug, culture 37 days old, conidia from sporodochium:
6-septate, rare, 38.8 by 4.15μ (1).
5-septate, 15 per cent, 40 by 4μ average (10), 36.5 to 45 by 3.32 to 4.15μ.
4-septate, 29.4 per cent, 38.6 by 4μ average (10), 31.5 to 41.5 by 3.32 to 4.15μ.
3-septate, 24.5 per cent, 36.6 by 4μ average (10), 33.2 to 41.5 by 3.32 to 4.15μ.
Small 3 septate, 6.9 per cent, 19 by 2.8μ average (10), 16.6 to 25 by 2.5 to 3.32μ.
2-septate, 2.6 per cent, 17 by 2.9μ average (3), 15 to 20 by 2.9μ.
1-septate, 5.2 per cent, 13.4 by 2.7μ average (10), 8.3 to 16.6 by 1.7 to 3.32μ.
0-septate, 16.4 per cent, 7.5 by 2.8μ average (10), 5.8 to 10 by 1.7 to 3.32μ.

On Melilotus stems, culture 26 days old, conidia from pionnotes:
From thick pionnotes at moist bottom of tube: 3-septate, 38 per cent; 0-septate, 62 per cent.
From thin pionnotes on dry upper portion: 3-septate, 64.8 per cent; 2-septate, 2.3 per cent; 1-septate, 5.3 per cent; 0-septate, 27.6 per cent.
3-septate, 39.3 by 3.2μ average (10), 24 to 41.6 by 2.8 to 3.6μ.
1-septate, 16 by 2μ (1).
Chlamydospore 6.4 by 6.4μ (1).

On onion-agar tube, culture 20 days old, conidia from pionnotes:
5-septate, 6.5 per cent, 45 by 4.4μ average (10), 41.5 to 50 by 3.7 to 5μ.
4-septate, 20.4 per cent, 45.2 by 4.23μ average (10), 41.5 to 50 by 3.32 to 4.56μ.
3-septate, 66.1 per cent, 45.3 by 4.15μ average (10), 41.5 to 48 by 3.32 to 4.56μ. 1-septate, 2 per cent, 13.7 by 3.32μ average (10), 10 to 18.3 by 2.9 to 4.56μ. 0-septate, 5 per cent, 8.3 by 3μ average (10), 5 to 11 by 2 to 4.56μ.

On steamed rice, culture 37 days old, conidia from dry sporodochium:
4-septate, 3 per cent, 47.5 by 4.4μ average (2), 47 to 48 by 4.25 to 4.5μ.
3-septate, 89 per cent, 39 by 3.73μ average (15), 35 to 43 by 3.5 to 4.5μ (30 by 3.47 by 3.5)μ.
1-septate, 1 per cent.
0-septate, 7 per cent.

On 20 per cent dextrose-agar plate, culture 50 days old, conidia from pionnotes:
4-septate, 6.7 per cent.
3-septate, 64.2 per cent.
1-septate, 0.6 per cent.
0-septate, 28.5 per cent.
Average of all media:
6-septate, 39.8 by 4.15μ average (1).
5-septate, 42.1 by 4.1μ average (29), 36.5 to 50 by 3.2 to 5μ.
4-septate, 39.9 by 3.8μ average (55), 31.5 to 53 by 3.2 to 4.56μ.
3-septate, 38.5 by 3.7μ average (93), 24 to 48 by 2.4 to 4.56μ.
Small 3-septate, 19 by 2.8μ average (10), 16.6 to 25 by 2.5 to 3.32μ.
2-septate, 18.1 by 3.2μ average (4), 15 to 21.6 by 2.9 to 4.15μ.
1-septate, 14 by 3μ average (22), 8.3 to 18.3 by 1.7 to 4.56μ.
0-septate, 7.9 by 2.8μ average (25), 5 to 11.6 by 1.7 to 4.56μ.

Of the 10 cultures studied critically, 1324 (Delta, Colo.), 1626–b (Walla Walla, Wash.), 1455–d (Walla Walla, Wash.), 1495 (Delta, Colo.), and 1407 (Stockton, Calif.) were found nonpathogenic. No. 1324 is *F. vasinfectum* Atk., 1626–b is *F. oxysporum* (Schlecht.) Wr., 1455–d is *F. bulbigenum* Cke. et Mass., 1495 is *F. martii*, Appel et Wr. var. *minus* Sherb., and 1407 may be *F. malli* Tauben. No positive diagnosis has yet been made of 1407 which belongs to group Martiella because the fungus has consistently refused to produce macrospores. Cultures 840–b and 1624–b′ are *F. moniliforme* Sheld. and are pathogenic sometimes. (See Table 4.)

During the course of the identification of the organisms nine authentic cultures were obtained through the courtesy of H. W. Wollenweber. These and 15 additional authentic cultures were used in inoculation work with the results shown in Table 6.

Fusarium cepae rots bulbs, whereas inoculations with cultures of *F. aurantiacum* and *F. sclerotioides* proved negative; this table and Table 4 indicate that the Fusaria which cause bulb rot are specialized pathogenes. Other members of the form genus Fusarium which occur commonly in the soil, or which occur on rotted bulbs in association with bacteria or the true pathogenes, fail to produce rot under experimental conditions.

TABLE 6.—*Inoculation with authentic Fusarium cultures*

Culture No.	Name of organism	Where obtained[a]	Number of inoculations	Number of bulbs decayed
1592	F. batatatis	S	3	0
1525	F. coeruleum	S	3	0
1594	F. conglutinans	J	3	0
1523	F. discolor	S	3	0
1506	F. eumartii	S	3	0
1611	F. hyperoxysporum	H	3	0
1593	F. hyperoxysporum	H	3	0
1533	F. hyperoxysporum	H	3	0
1518	F. lycopersici	S	3	0
1505	F. oxysporum	S	3	0
1507	F. radicicola	S	5	[b] 2
1534	F. solani	S	3	0
1520	F. sulphureum	S	3	0
1522	F. vasinfectum	S	3	0
1628	F. redolens	W	2	0
1629	F. orthoceros	W	2	0
1630	F. sclerotium	W	2	0
1631	F. sclerotioides	W	2	0
1632	F. zonatum	W	4	0
1633	F. euoxysporum	W	2	0
1634	F. bulbigenum	W	2	0
1635	F. aurantiacum	W	2	0
1636	F. orthoceros var. longius	W	2	0
1660	F. malli	N	5	[c] 2

[a] H, L. L. Harter; J, Helen Johann; N, A. G. Newhall at Cornell; S, Michael Shapovalov; W, H. W Wollenweber.
[b] Slight decay, but not typical rot.
[c] Rot+bacteria.

SUMMARY

A bulb rot of onions caused by Fusarium species occurs widely in the field and after harvest in transit and in storage.

Red, yellow, and white varieties are equally susceptible. Decay was readily produced when bulbs were inoculated in wounds.

The Fusaria which cause onion bulb rot apparently are specialized pathogenes attacking only Allium species.

Several species of Fusarium were found to cause identical symptoms. Most of these species belong to section Elegans. In this paper are described *F. zonatum* (Sherb.) Wr. forma 2, nova forma and *F. cepae* (Hanzawa) emend. (non *F. cepae* (Hanzawa) emend. Walker and Tims). Another species of this section which is always pathogenic is *F. zonatum* (Sherb.) Wr. forma 1.

Fusarium bulbigenum Cke. et Mass. of section Elegans is not consistently pathogenic. *F. oxysporum* (Schlecht.) Wr. and *F. vasinfectum* Atk. are not pathogenic.

Fusarium moniliforme Sheld. of section Liseola is pathogenic at times.

Not all Fusaria isolated from decaying bulbs are pathogenic in pure culture. Some of them cause symptoms similar to bulb rot when in association with bacteria. Most of these belong to section Elegans and to section Martiella. Some belong to sections Discolor and Liseola.

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THE ALTERNATE HOSTS OF CROWN RUST, PUCCINIA CORONATA CORDA[1]

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INTRODUCTION

As has been shown previously (*9*, *4*),[3] many species of the genus Rhamnus are susceptible to crown rust (*Puccinia coronata* Corda) and play an important rôle in the dissemination of this disease in America. The universal presence of the uredinial stage of this rust in certain regions where no Rhamnus is found or only resistant species occur led the writer to a further study of the range of the aecial hosts of this fungus.

European investigators have been interested in the alternate hosts of crown rust since 1866 when De Bary (*3*) discovered that *Rhamnus cathartica* L. harbored the aecial stage. An earlier résumé (*9*) of the literature on the alternate hosts of *Puccinia coronata* and their ability to spread rust in the open (*4*) makes unnecessary a review in this paper.

The reaction of 16 species and 1 variety of Rhamnus to crown rust, the testing of 4 genera other than Rhamnus, and the determination of the rôle of *R. cathartica* L. and *R. lanceolata* Pursh. in initiating local and general epidemics of *Puccinia coronata* in the upper Mississippi Valley are considered in this paper.

MATERIALS AND METHODS

During the past six years studies have been made in the greenhouse and laboratories of the Iowa Agricultural Experiment Station at Ames, Iowa, to determine the reaction of 16 species and 1 variety of Rhamnus, and 5 species of 4 other genera, to crown rust. Succulent young leaves were inoculated with germinating teliospores from *Avena sativa* L., *Calamagrostis canadensis* (Michx.) Beauv., *C. purpurascens* R. Br., *Festuca elatior* L., and *Notholcus lanatus* (L.) Nash. (*Holcus lanatus* L.). The species of Rhamnus and other genera were exposed to infection by placing them in a moist chamber with teliospore-laden straw. The plants, straw, and sides of the chamber were then wet by a fine spray. In cases where scant spore material was available, the spores were germinated on the straw in Petri dishes as described by Melhus and Durrell (*8*). Later, the sporidia were washed off and sprayed onto the plants with an atomizer. If the chamber showed signs of becoming dry before the close of the incubation period, it was opened and sprayed again. This

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[2] The writer wishes to express to I. E. Melhus, J. C. Gilman, and H. B. Humphrey his appreciation for much assistance in the preparation of the manuscript, and to the last named also his thanks for the collection of the field data on *Rhamnus frangula* L.

[3] Reference is made by number (italic) to "Literature cited." p. 969.

method successfully induced infection. At the end of two days the plants were removed to the greenhouse bench, where they were observed for the appearance of rust.

SOURCE AND CARE OF ALTERNATE HOSTS

Five additional native and two European species of Rhamnus have become available for alternate-host studies since 1922. Species of Ceanothus, Berchemia, and Zizyphus, of the family Rhamnaceae, together with two species of Lepargyrea, of the family Eleagnaceae, have been studied to determine their reaction to germinating teliospores of *Puccinia coronata*.

Rhamnus dahurica Pallas is a cultivated shrub indigenous to the territory between India and northern Asia. Its bark is used in the manufacture of Chinese green indigo. Because of its extreme hardiness, this shrub is becoming common for landscaping in certain sections of America where the winters are severe. The material used here was grown from seed collected on the campus at Ames, Iowa.

Rhamnus ilicifolia Kellogg is native to southern and middle California, and is especially abundant on Angel Island, in San Francisco Bay, where it often reaches a height of 10 to 12 feet. It is very similar to *R. crocea*, but differs from the latter in its arboreous habit, gray bark, and larger leaves and fruit. This material was obtained in California.

Rhamnus nevadensis Nelson is indigenous to Nevada. It is very similar to *R. californica* Esch., but has noncoriaceous leaves and short calyx lobes. It seems much hardier under Iowa conditions. The one plant available in this investigation was found in Nevada.

Rhamnus pachyphylla Heller, native to California, has a limited distribution. Its leaf is thick and leathery with an entire margin. *R. pinetorum* Heller also has a rather limited distribution in California.

Rhamnus rubra Greene is listed by Trelease (*14*) as a variety of *R. californica*. Greene (*6, p. 160*), however, thinks it a good species, since its seeds are only half as large as those of *R. californica* and also are decidedly narrowed at the base. The plants used in these experiments had bark of a deeper red than those of *R. californica*. This species is common in California, especially in Butte County between Upper Soda Springs and Shasta Retreat. The writer is indebted to A. A. Heller for the collection of *R. pachyphylla*, *R. pinetorum*, and *R. californica*.

Rhamnus tinctoria Waldst. and Kit. is native to Europe and Asia Minor, where it is used in the manufacture of dye.

Berchemia scandens (Hill) Trel. is a climbing plant growing in damp soils from Virginia to Missouri and southward. The stems are tough and lithe, hence the popular name, supple-jack. This material was collected in southern Alabama.

Ceanothus americanus L., commonly called New Jersey tea, grows in dry woodlands, along gravelly shores and sandy knolls, from central Maine to western Ontario and southward through Iowa. The material employed in these studies was collected 3 miles north of Ames, Iowa, on virgin prairie land.

Lepargyrea argentea Greene is widely distributed in the northeastern part of the United States and Canada, extending southward

to northern New Mexico in the Rocky Mountain region. *L. canadensis* Greene is even more extensive in distribution than *L. argentea*. It extends across southern Canada and up to Alaska. In the Rocky Mountain region, however, temperature seems to limit its southern distribution, as this shrub could be found only at an elevation in excess of 7,500 feet near Bergen Park, in Colorado. The materials used in these investigations were collected at Pagosa Spring and Bergen Park, Colo.

Zizyphus lycioides Gray, a spiny shrub with zigzag branches, is native to the southern part of the United States. The material used was collected in southern Alabama.

Considerable difficulty was experienced at first in overwintering these plants. As an extremely succulent growth was needed for inoculation during six weeks in the spring, the question arose as to the best method of maintaining these plants throughout the remainder of the year. Four methods, all unsatisfactory at Ames, were as follows: (1) Maintenance under greenhouse conditions throughout the year; (2) heeling-in during the winter out of doors and transferring to the greenhouse in the spring; (3) packing in moss and keeping in cold storage during the winter; (4) cuttings.

Two methods of obtaining succulent spring growth were successfully used at Ames. In the first, the plants were set in 12-inch pots placed outdoors in June, and set 3 inches below the ground line. They were allowed to harden off in the fall until defoliated, after which they were covered with 6 inches of straw and allowed to remain outside until the first of March. They were then kept in a cold room (45° to 55° F.) for three weeks and subsequently placed on the greenhouse bench (temperature 65° to 70°). It seems important that these plants be started slowly when brought into the greenhouse, as in previous years over 50 per cent of them died when taken directly from the outside into a heated room.

A second satisfactory method was to sow Rhamnus seed in the greenhouse in November. Seeds of *R. dahurica*, *R. lanceolata*, *R. cathartica*, *R. californica*, *R. purshiana*, and *R. alnifolia* germinated strongly if gathered in the fall and sown before the fruit became dry.

After the plants had become established on the greenhouse bench in the spring, two methods of obtaining succulent growth were used: (1) The plants were watered daily with liquid manure and the temperature raised to 75° to 80° F.; (2) the seedlings, which had been maintained under greenhouse conditions, were severely pruned and then forced by means of fertilizer and heat.

SOURCE AND CARE OF TELIOSPORES

During 1923, teliospores from *Avena sativa* L. were collected from four places in Iowa and Illinois. These had a low percentage of germination. Those collected at Ames and Masonville, Iowa, during 1924, germinated with greater vigor.

Teliospores were collected from *Calamagrostis canadensis* in Iowa, Wisconsin, and Canada. Those from Iowa germinated between 90 and 100 per cent, but the other collections had only a weak germination. Teliospores were collected at Eldora Springs, Colo., from *C. purpurascens*.

A collection of teliospores from *Festuca elatior* was made at Ames, Iowa. Teliospores were collected from greenhouse cultures of *Notholcus lanatus* but germinated poorly.

The germination of teliospores in the spring was influenced by the method of overwintering the host plants. Those from *Avena sativa*, *Festuca elatior*, and *Notholcus lanatus* germinated most satisfactorily when the host plant was left in the field and the spore material collected in the spring, while those on *Calamagrostis canadensis* germinated equally well whether collected in the fall and wrapped in cheesecloth or obtained from the host plant in the spring.

TYPES OF INFECTION OF ALTERNATE HOSTS

Successful attempts to infect Rhamnus species developed the following four types of aecial infection: (1) Necrotic areas, no sporulation; (2) normal pycnia, no aecia; (3) normal pycnia, and few aecia; (4) normal pycnia and aecia.

In type 1 the germ tube of the sporidium caused etiolated, light yellow areas, usually surrounding a dead center with no form of sporulation. In type 2, there was normal pycnial development but little or no evidence of aecia. Type 3 showed normal pycnia but few aecia. Type 4 was characterized by profuse development of normal pycnia and aecia.

Growth factors, such as relative humidity and temperature, played an important part in determining whether an infection of type 3 or 4 would develop. A high humidity and a temperature of about 20° C. caused profuse development of aecial cups on susceptible Rhamnus species. Inherent rather than environmental causes were probably more responsible in determining whether aecial infection was of type 2 or 3.

THE ALTERNATE HOSTS OF CROWN RUST

It has been shown earlier (*9*) that the uredinial hosts of *Puccinia coronata* are not restricted to one genus. Whether the alternate host of crown rust was limited to the genus Rhamnus has not previously been known. To determine this, the following genera were exposed to infection from teliospores of crown rust: Berchemia, Ceanothus, Leparagyrea, Rhamnus, and Zizyphus.

THE GENUS RHAMNUS

The results obtained by exposing 16 species and 1 variety of Rhamnus to crown-rust infection 364 times are presented in Table 1. Teliospores from *Avena sativa*, *Calamagrostis canadensis*, and *Festuca elatior* were used, as these gramineous hosts are generally present throughout the main oat-growing sections of the upper Mississippi Valley.

TABLE 1.—*Response of Rhamnus species to inoculation with teliospores of Puccinia coronata from various grasses, in the years 1919 to 1924, inclusive*

Rhamnus species	Teliospores from—	Times inoculated	Types of infection				
			None	Flecks	Pycnia	Few aecia	Normal
Alnifolia	Avena sativa	12	7	1		4	
Do	Calamagrostis canadensis	15	2	1			12
Do	Festuca elatior	2	1				1
Californica	Avena sativa	13	4			9	
Do	Calamagrostis canadensis	18	1	1	7	8	1
Do	Festuca elatior	4	2		1		1
Californica var. tomentella	Avena sativa	7	2			2	3
Do	Calamagrostis canadensis	13	3	4	6		
Do	Festuca elatior	4	2		2		
Caroliniana	Avena sativa	13	7		3	1	2
Do	Calamagrostis canadensis	11	5	2	4		
Do	Festuca elatior	2				2	
Cathartica	Avena sativa	26	2				24
Do	Calamagrostis canadensis	24	14	5	3	2	
Do	Festuca elatior	5					5
Crocea	Avena sativa	1				1	
Do	Calamagrostis canadensis	6					6
Do	Festuca elatior	1				1	
Dahurica	Avena sativa	13	5	1	3	4	
Do	Calamagrostis canadensis	6	5		1		
Do	Festuca elatior	2	2				
Frangula	Avena sativa	17	8	7	2		
Do	Calamagrostis canadensis	28	23	3	2		
Do	Festuca elatior	3	3				
Ilicifolia	Avena sativa	4	2				2
Do	Calamagrostis canadensis	3	3				
Do	Festuca elatior	1		1			
Lanceolata	Avena sativa	11	2				9
Do	Calamagrostis canadensis	24	5	1		4	14
Do	Festuca elatior	3					3
Nevadensis	Avena sativa	1	1				
Do	Calamagrostis canadensis	0					
Do	Festuca elatior	0					
Pachyphylla	Avena sativa	5	1		3		1
Do	Calamagrostis canadensis	4			4		
Do	Festuca elatior	1		1			
Pinetorum	Avena sativa	3	1				2
Do	Calamagrostis canadensis	2				2	
Do	Festuca elatior	1	1				
Purshiana	Avena sativa	8	3	3	2		
Do	Calamagrostis canadensis	10	7	2	1		
Do	Festuca elatior	3	3				
Rubra	Avena sativa	5				4	1
Do	Calamagrostis canadensis	6				6	
Do	Festuca elatior	2	1			1	
Smithii	Avena sativa	7	1			2	4
Do	Calamagrostis canadensis	10	1				9
Do	Festuca elatior	1				1	
Tinctoria	Avena sativa	1				1	
Do	Calamagrostis canadensis	1			1		
Do	Festuca elatior	1	1				

When inoculated with teliospores from *Avena sativa*, it was found that *Rhamnus californica* var. *tomentella*, *R. caroliniana*, *R. cathartica*, *R. ilicifolia*, *R. lanceolata*, *R. pachyphylla*, *R. pinetorum*, *R. rubra*, and *R. smithii* were susceptible, producing type-4 infection, and that *R. alnifolia*, *R. californica*, *R. crocea*, *R. dahurica*, and *R. tinctoria* produced a type-3 infection (figs. 1 and 2). *R. frangula*, *R. nevadensis*, and *R. purshiana* showed a marked resistance to this physiologic form of rust. *R. dahurica* probably possesses inherent resistance (fig. 2), as the aecial sori were small, and contained only a few cluster cups. More inoculations are necessary to determine the true response of *R. nevadensis*, as only one plant was available in the experiments here recorded. When inoculated with teliospores from *Calamagrostis canadensis*, *R. alnifolia*, *R. californica*, *R. crocea*, *R. lanceolata*, and *R.*

smithii were infected normally. In addition, *R. cathartica*, *R. pinetorum*, and *R. rubra* may function as alternate hosts for this form, as they produced a type-3 infection. The other eight species of Rhamnus showed a marked resistance.

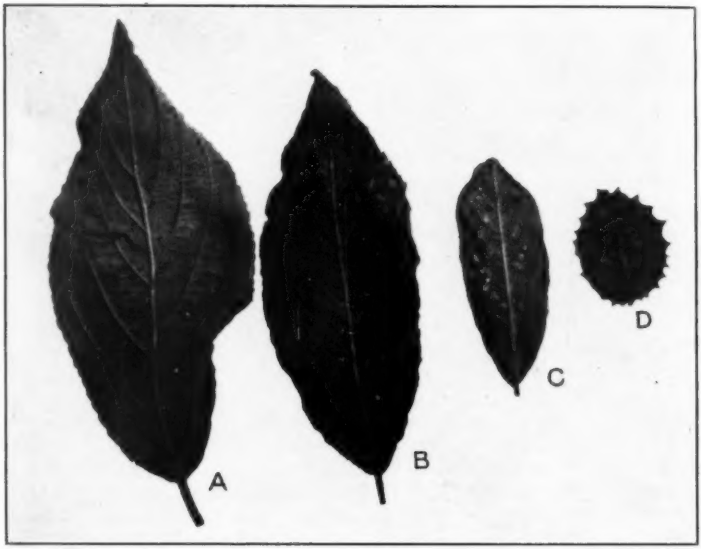

FIG. 1.—Infection from *Avena sativa* on Rhamnus species. A, *Rhamnus alnifolia;* B, *R. caroliniana;* C, *R. rubra;* D, *R. ilicifolia*

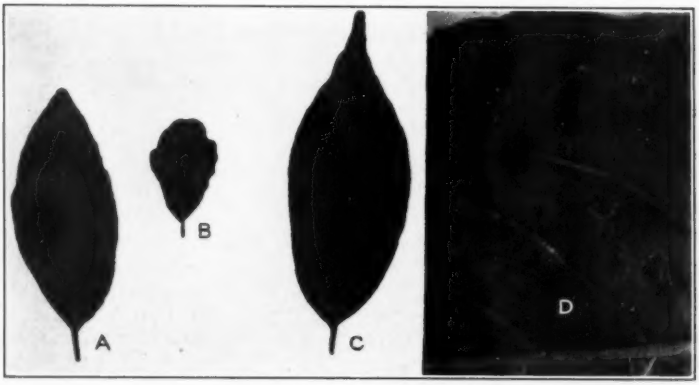

FIG. 2.—Infection from *Avena sativa* to Rhamnaceae. A, *Rhamnus smithii;* B, *R. pinetorum;* C, *R. lanceolata;* D, *R. dahurica.* × 3

Teliospores from *Festuca elatior* caused normal infection on *Rhamnus alnifolia*, *R. californica*, *R. cathartica*, and *R. lanceolata*. On *R. caroliniana*, *R. crocea*, *R. rubra*, and *R. smithii* a few aecia were produced. Twelve inoculation trials on the seven remaining Rhamnus species

showed that *R. ilicifolia* produced pycnia and *R. pachyphylla* became flecked. Owing to the limited teliospore material, further investigation is necessary to determine the reponse of Rhamnus species to crown rust from *F. elatior*.

A limited inoculum of teliospores from *Notholcus lanatus* was transferred to *Rhamnus alnifolia*, *R. californica*, *R. californica* var. *tomentella*, *R. cathartica*, *R. caroliniana*, *R. crocea*, *R. frangula*, *R. lanceolata*, *R. purshiana*, and *R. smithii*. *R. lanceolata* produced pycnia. Further tests are necessary.

It should be noted that the reaction of the Rhamnus species to crown rust from *Festuca elatior* paralleled closely that resulting from the strain from *Avena sativa*. A greater divergence between the reaction of the *A. sativa* and *Calamagrostis canadensis* strains is apparent. The reaction of *R. caroliniana* to the strain from *A. sativa* is particularly noticeable, as this species was infected normally but twice in 13 attempts. The normal infection occurred during 1924, the most favorable year for obtaining infection in the six years of experimentation. Although *R. frangula* was inoculated 48 times and *R. purshiana* 21 times, both maintained a marked resistance to crown rust from the three gramineous hosts.

Many of the Rhamnus species contained some individuals which showed no infection and others which showed normal infection (Table 1). This variation probably was not always due to an inherent difference in the individuals, as some of the same plants developed no infection at one inoculation and normal infection during a subsequent attempt. The species were divided into susceptible and resistant groups. All that functioned as alternate hosts by producing aecia were classified as susceptible.

TABLE 2.—*Division of Rhamnus species into susceptible and resistant groups according to types of infection*

Inoculated with teliospores from—	Susceptible species							Resistant species						
	Number of species	Number of trials	Types of infection					Number of species	Number of trials	Types of infection				
			None	Flecks	Pycnia	Few aecia	Normal			None	Flecks	Pycnia	Few aecia	Normal
Avena sativa	14	121	34	2	9	28	48	3	26	12	10	4	0	0
Calamagrostis canadensis	[a]9	108	26	12	10	28	32	[a]7	63	43	7	13	0	0
Festuca elatior	[a]9	24	6	0	1	7	10	[a]7	12	10	1	1	0	0
Total		253	66	14	20	63	90		101	65	18	18	0	0

[a] *Rhamnus nevadensis* was exposed to infection only with teliospores from *Avena sativa*.

The production of a single aecium was considered indicative of the inherent susceptibility of a species, and when further trials produced only negative results it was held to be due probably to the environmental conditions of the plant during inoculation or to possible nonviability of the teliospores. As shown in Table 2, the mode of each dispersion is at the normal class when inoculations were made with spores from *Avena sativa*, *Calamagrostis canadensis*, or *Festuca*

elatior, respectively. A total of 253 inoculations of the species classified as susceptible resulted in 187 positive and 66 negative reactions; 63 developed a few aecia. Ninety of the 187 positive reactions were of normal type.

Those species were classed as resistant which did not function as alternate hosts; that is, those which produced only a maximum development of pycnia, or produced only flecks, or no infections. In this class, 65 of a total of 101 inoculations, or over 64 per cent, were negative. It is obvious that it is increasingly more difficult to secure infection in the resistant than the susceptible class, a condition due probably to the inherent resistance of certain species of Rhamnus.

The maximum degree of infection obtained at any time on each species of Rhamnus is recorded in Table 3. As the importance of any Rhamnus species as an alternate host may depend on its degree of susceptibility under optimum conditions, the maximum degree of infection serves as an index of the possible danger from each particular species. Crown rust obtained from all of the three telial hosts developed aecia on *R. alnifolia*, *R. californica*, *R. californica* var. *tomentella*, *R. cathartica*, *R. crocea*, *R. lanceolata*, *R. rubra*, and *R. smithii*.

TABLE 3.—*Maximum infection on Rhamnus species, expressed in types of infection 1 to 4, after inoculation with teliospores from three grasses during the period 1919 to 1925, inclusive*

Inoculated with teliospores from—	R. alnifolia	R. californica	R. californica tomentella	R. caroliniana	R. cathartica	R. crocea	R. dahurica	R. frangula	R. ilicifolia	R. lanceolata	R. nevadensis	R. pachyphylla	R. pinetorum	R. purshiana	R. rubra	R. smithii	R. tinctoria
Avena sativa	3	3	4	4	4	3	3	2	4	4	[a]0	4	4	2	4	4	3
Calamagrostis canadensis	4	4	3	2	3	4	2	2	0	4	....	2	3	2	3	4	2
Festuca elatior	4	4	3	3	4	3	0	0	1	4	....	1	0	0	3	3	0

[a] 0 = no infection.

Figure 3 represents those species of Rhamnus which may function as alternate hosts for the physiologic forms of crown rust on *Avena sativa*, *Calamagrostis canadensis*, or *Festuca elatior*.

OTHER GENERA

Other genera of Rhamnaceae and Eleagnaceae were studied as alternate hosts of crown rust. The late Ellsworth Bethel, of Denver, Colo., found *Lepargyrea canadensis* and *L. argentea* bearing aecia when growing near gramineous hosts of crown rust. Arthur (*1*) exposed these two shrubs to teliospores from *Notholcus lanatus* and *Scolochloa festucacea* but obtained no infection.

Lepargyrea canadensis, L. argentea, Ceanothus americanus, Berchemia scandens, and *Zizyphus lycioides* were inoculated in 1921 with teliospores from *Avena sativa, Calamagrostis canadensis, C. purpurascens,* and *Festuca elatior* (Table 4). On *L. canadensis,* inoculated with teliospores from *C purpurascens,* a heavy normal infection developed (fig. 4). All other results with this gramineous host were negative. The results on Lepargyrea were verified by cultures grown under controlled conditions at Denver, Colo., by I. W. Clokey during the summer of 1922. Unfortunately, no teliospores were available in 1923 with which to inoculate Ceanothus, Berchemia, and Zizyphus.

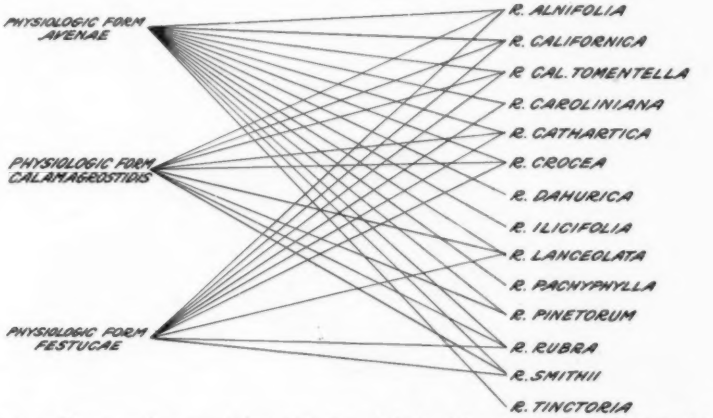

FIG. 3.—Diagrammatic representation of the alternate hosts of three physiologic forms of *Puccinia coronata*

TABLE 4.—*Reaction to Puccinia coronata of species in genera other than Rhamnus*

Inoculated with teliospores from—	Species inoculated					
	Number of inoculations on each species	Lepargyrea argentea	Lepargyrea canadensis	Ceanothus americanus	Berchemia scandens	Zizyphus lycioides
Avena sativa	3	0	0	0	+	0
Calamagrostis canadensis	3	0	0	0	0	0
Calamagrostis purpurascens	4	0	+	−	−	−
Festuca elatior	2	0	0	0	0	0

0 = no infection. + = infection. − = not tried.

In July, 1924, aecia were collected on *Lepargyrea canadensis* near Bergen Park, Colo., and aeciospores from these were transferred to *Avena sativa* at Ames, Iowa. Although urediniospores were produced on oats, the results are not conclusive because the aecia were not produced under controlled conditions. *L. argentea* is listed by Arthur (*2, p. 313*) as an alternate host of crown rust, but the writer was unable to obtain infection through inoculation with teliospores from *A. sativa, Calamagrostis canadensis, C. purpurascens,* or *Festuca elatior,* at Ames, Iowa.

Berchemia scandens was inoculated on May 5, 1924, with teliospores from *Avena sativa*. By May 19, pycnia had developed and two days later a few aecia were noted. This experiment was repeated, and four inoculated plants produced a few aecia. *B. scandens* may function as an alternate host for *Puccinia coronata*, although it shows some degree of resistance, as indicated by the small aecia with few cups.

FIG. 4.—*Lepargyrea canadensis* showing aecia after inoculation with teliospores from *Calamagrostis purpurascens*

THE EFFECT OF RHAMNUS SPECIES ON PHYSIOLOGIC FORMS OF P. CORONATA

As a heteroecious rust like *Puccinia coronata* completes its life cycle on two widely different plants, the question of the possible effect of the alternate host on the biology of the parasite frequently has arisen. Whether the alternate host stimulates or invigorates the fungus in some manner, or changes its biologic response, has not been known. Some light is thrown on the latter phase in this paper.

The range of gramineous hosts of the physiologic forms of crown rust has been previously reported (*9*). In an earlier report (*9*) and in the present paper, the reaction of 16 Rhamnus species to crown rust is recorded. In order to complete the chain of evidence, the range of gramineous hosts of the aeciospores from Rhamnus should be determined.

Inoculating with teliospores from *Avena sativa*, aecial infection was secured on *Rhamnus caroliniana*, *R. cathartica*, *R. dahurica*, *R. ilicifolia*, *R. lanceolata*, *R. pinetorum*, *R. rubra*, and *R. smithii*. *Achyrodes aureum*, *Avena sativa*, *Calamagrostis canadensis*, *Festuca elatior*, and *Notholcus lanatus* were inoculated with aeciospores from each infected species of Rhamnus. Aeciospores from all eight Rhamnus species caused infection on *Avena sativa*. All results are shown in Table 5.

TABLE 5.—*Gramineous host range of Puccinia coronata avenae, determined by inoculation with aeciospores from eight Rhamnus species*

Species	Gramineous hosts				
	Achyrodes aureum	Avena sativa	Calamagrostis canadensis	Festuca elatior	Notholcus lanatus
R. caroliniana	+*	+			+
R. cathartica	+	+		+	+
R. dahurica		+			
R. ilicifolia		+	+	+	
R. lanceolata	+	+	+	+	+
R. pinetorum		+			+
R. rubra	+	+			+
R. smithii		+			+

* +=infection.

In some cases, the number of aeciospores was limited; hence the negative results of Table 5 are not conclusive. This was true particularly of *Rhamnus dahurica*, *R. ilicifolia*, and *R. pinetorum*. Inoculum from *R. caroliniana*, *R. cathartica*, *R. lanceolata*, *R. pinetorum*, *R. rubra*, and *R. smithii* produced urediniospores on *Notholcus lanatus*. Urediniospores were produced on *Calamagrostis canadensis* inoculated with aeciospores from *R. ilicifolia* and *R. lanceolata*. *Achyrodes aureum* has been shown to be a host for five physiologic forms of *Puccinia coronata*, two physiologic forms of *P. graminis* and *P. dispersa*, *P. montanensis*, and *P. poarum*. Urediniospores were produced on this grass through inoculation with aeciospores from *R. caroliniana*, *R. cathartica*, *R. lanceolata*, and *R. rubra*.

Rhamnus lanceolata, which had been inoculated with teliospores from *Calamagrostis canadensis*, produced abundant urediniospores on *Avena sativa* in nine trials conducted over a period of three years.

CORRELATION OF LABORATORY AND FIELD RESULTS

Although Rhamnus species may become infected with crown rust under greenhouse conditions, the importance of the alternate hosts of this rust depends on such factors as prevalence and distribution of the species, the quantity of infection, together with the methods of infection, rate of dissemination of their aeciospores, and the resulting damage to the oat crop.

RHAMNUS CATHARTICA IN THE UPPER MISSISSIPPI VALLEY

Through the cooperation of the State leaders of barberry eradication and their field assistants with the plant pathology section of the Iowa Experiment Station, a preliminary survey for the locations of common buckthorn (*Rhamnus cathartica*) was made. In representative areas, comprising less than one-twentieth of the total area in each of the eight upper Mississippi Valley States, a total of 1,021 plantings of *R. cathartica* was found during 1923 and 1924. Of these plantings, 282 were rural and 739 urban. They contained 93,041 counted bushes besides 44,395 feet of hedge where the bushes were not counted. Many wild areas of escaped bushes found in Illinois, Wisconsin, and Montana were not included in this count, for the bushes were too numerous to estimate.

It readily can be seen that with this tremendously large number of shrubs in a relatively small area, a serious, though local, annual damage to the oat crop may result if these shrubs become infected. Since the aecial infection affecting the oat crop could function only a short time in the spring, the percentage of bushes infected was tabulated for the months of May and June, 1923. Nearly 80 per cent of all bushes reported during this period were infected, while those reported from the farmsteads were uniformly infected. In 1924, about 50 per cent of the bushes located during this period were infected. For the past nine years, under Iowa conditions, aecial infection of *Puccinia coronata* developed on *Rhamnus cathartica* and *R. lanceolata* before uredinia were present on *Avena sativa* (Table 6).

TABLE 6.—*Time of appearance of crown-rust uredinia on Avena sativa and aecia on Rhamnus cathartica and R. lanceolata in Iowa for the period 1916 to 1924, inclusive*

Stage in life cycle	Host	Year				
		1916	1917	1918	1919	1920
Aecial	Rhamnus cathartica	May 7	June 30	Apr. 30	May 26	May 15
Aecial	Rhamnus lanceolata	May 22	.....	June 6	May 15	May 20
Uredinial	Avena sativa	June 2	July 6	July 6	June 6	June 12

Stage in life cycle	Host	Year			
		1921	1922	1923	1924
Aecial	Rhamnus cathartica	May 16	May 14	May 21	May 28
Aecial	Rhamnus lanceolata	May 16	May 21	May 23	May 7
Uredinial	Avena sativa	May 22	June 14	June 16	June 16

The earliest appearance of aecia during this period was found on *Rhamnus cathartica* on April 30, and the latest on June 30. The time of the appearance of infection depends somewhat on climatic factors, especially moisture and temperature. *R. lanceolata* varied in time of observed infection from May 7 to June 6. Uredinia usually appear on oats about seven days after visible outbreak of the disease on the alternate host. However, this period was increased greatly in 1918 by the hot, dry weather intervening between aecial and uredinial infection.

In order to prove further that buckthorn was responsible for the initial infection in Iowa, a detailed survey of the State was made during May and June, 1923 and 1924. If wind-blown spores from the south started the infection in Iowa, a more or less uniform rust spread should have appeared first across the southern part of the State. However, during this time, many local areas or concentric zones of crown-rust infection of oats were observed scattered over the State previous to a general infection. Either *Rhamnus cathartica* or *R. lanceolata* was found near the center of each of these areas. The degree of infection varied from a trace on the outer edge of these concentric zones to 30 per cent near the center. These local infection centers appeared simultaneously in northern and southern Iowa. The areas were small at first, remained constant for 7 to 10 days, and then expanded rapidly. This probably was due partly to the limited area over which aeciospores cause infection (*4*). It is probable that infection from rural plantings of Rhamnus is more likely to spread than that from city plantings. This belief is supported by the fact that the centers of all these local infection areas were on farmsteads.

A detailed study of the effect of crown rust on yield was made at Winthrop in Buchanan County, Iowa, where the initial infection was a hedge of *Rhamnus cathartica* adjoining an oat field. The oats were not cut, as many of the plants failed to head. Oats in adjoining sections yielded 10 bushels per acre, while the average yield of more remote sections of the same township was 50 bushels per acre.

RHAMNUS FRANGULA

As previously stated, the maximum infection on *Rhamnus frangula* under controlled conditions gave only pycnia production in 4 out of 48 trials. It seemed desirable to ascertain whether the species maintained this relative resistance under field conditions. During 1923 and 1924, ten plantings or thickets of *R. frangula* in the New England States, ranging from a few to more than 2,000 bushes were inspected for aecial infection. In four of these cases, where *R. frangula* and *R. cathartica* were growing side by side, and often interwoven, aecial infection was found abundantly on *R. cathartica* but was absent on *R. frangula*. Likewise no aecia were found on the other six plantings of *R. frangula*. *R. frangula* bushes, growing on the campus at Ames, Iowa, have produced no aecia during the past eight years of observation, while adjoining bushes of *R. cathartica* were infected annually.

RHAMNUS PURSHIANA

Under controlled conditions, *Rhamnus purshiana*, like *R. frangula*, proved resistant to crown rust. It is a well-known fact that this species produces abundant aecia under natural conditions, although

it remains to be discovered just what, if any, relationship this aecial infection bears to *Puccinia coronata* on *Avena sativa*.

In June, 1922, at New Amsterdam, B. C., the late Ellsworth Bethel succeeded in infecting *Calamagrostis* sp. by inoculating with aeciospores taken from *Rhamnus purshiana* grown in the open.

DISCUSSION

In Europe, crown rust (*Puccinia coronata*) has been divided by Klebahn (*7*) into two species, *P. coronata* and *P. coronifera*. Eriksson (*5*) and Mühlethaler (*10*) are in agreement with Klebahn that *Rhamnus frangula* acts as a differential host for *P. coronata* and *P. coronifera*. Eriksson (*5*) recognizes Barclay's *P. coronata* var. *himalensis* as still further distinct, since it produces aecia on *R. dahurica*. Dietel makes a valid species of this form, and Mühlethaler (*11*) recognizes it, but suggests that it may be *P. coronifera*. These investigators, then, have employed the several species of Rhamnus as indicators of different species of rust. In other words, strong emphasis has been placed on the physiologic reaction of the alternate host. Treboux (*12*), on the other hand, fails to find the marked differential reaction of Rhamnus species reported by Klebahn (*7*), Eriksson (*5*) and Mühlethaler (*11*).

Still further, here in America, Melhus, Dietz, and Willey (*9*) have presented infection experiments suggesting lack of marked differential action of *Rhamnus cathartica* and *R. frangula*. They found that teliospores from *Avena sativa* produced pycnia on *R. frangula* and that the 10 species considered by them showed different degrees of susceptibility ranging from flecking to the normal production of aecia. This varied host behavior led these authors to question the wisdom of founding a species of Puccinia on the basis of its alternate-host reaction.

In these further studies, comprising inoculation trials extending over four years and involving 16 species of Rhamnus, a wealth of additional data has been obtained bearing directly on the reaction of Rhamnus species to crown rust. It has been shown again that *R. frangula* is not immune from infection when inoculated with teliospores from *Avena sativa* and *Calamagrostis canadensis*. The development of pycnia was obtained on four different occasions. Infection is very dependent on the state of development of the leaf tissues and on the environmental conditions. It is quite conceivable that, if infection can be induced in the greenhouse, it also may be found under certain conditions in the open. It should be noted, however, that *R. frangula* is much more resistant than *R. cathartica* and *R. lanceolata*, although neither Klebahn (*7*), Eriksson (*5*), nor Mühlethaler (*10*, *11*) noted any variation in the degree of susceptibility of the two first named species.

Up to the present writing, teliospores of *Puccinia coronata* from *Avena sativa*, *Calamagrostis canadensis*, *Festuca elatior*, and *Notholcus lanatus* have been applied as inoculum to species of Rhamnus. Infection on *R. frangula* has resulted only through teliospores from *A. sativa* and *C. canadensis*. None has developed through teliospores from *F. elatior* and *N. lanatus*, probably because of the fewer trials with spores from the two last mentioned hosts and the added difficulty of securing viable teliospores from Festuca and Notholcus.

Further evidence showing that the alternate-host range of the American crown rust differs from that found in Europe is apparent in the reaction of *Rhamnus dahurica*. Barclay describes a variety of *Puccinia coronata* as *P. coronata* var. *himalensis* on *Agrostis himalensis*. Later, Eriksson transferred this variety to *R. dahurica* and subsequently Dietel raised Barclay's variety to specific rank, naming it *P. himalensis*. Mühlethaler subsequently recognized this species, but suggested that it might be synonymous with *P. coronifera* Kleb. As shown in Table 1, this method of differentiation is questionable where teliospores from *Avena sativa* produced aecia on *R. dahurica*, which, in turn, was able to produce urediniospores on *A. sativa* (Table 5). These data show that *R. dahurica* can not act as a differential host for *P. himalensis* (Barcl.) Diet., since teliospores from *A. sativa* also can produce aecia on this alternate host.

Another very significant point bearing on the reaction of Rhamnus species to infection is the preference manifested by crown rust taken from different gramineous hosts. As shown in Table 1, *Puccinia coronata* from *Avena sativa* infects most readily *R. cathartica* and *R. lanceolata*. *R. alnifolia* is most susceptible to the Calamagrostis form taken from *C. canadensis*. However, in each case, different degrees of infection were obtained on at least 15 species. At the same time, *R. alnifolia* has consistently shown resistance to the *A. sativa* form. In the case of *R. caroliniana*, the differential reaction of the two sources of crown rust is even more marked. Teliospores from *A. sativa* gave normal infection, while those from *C. canadensis* showed only pycnial development.

Although the reaction of crown rust from all the gramineous hosts has not been fully studied up to the present writing, the data available indicate clearly that certain species of Rhamnus are markedly resistant to some and susceptible to others. In other words, a physiologic form has its alternate-host range as well as its gramineous host range. Clearly, then, when the reaction of additional physiologic forms has been studied, still further overlapping and extension of alternate-host ranges should be discovered. As shown earlier, crown rust from *Avena sativa* was transferred for the first time to *Berchemia scandens*, one of the Rhamnaceae.

An additional extension of the alternate-host range of crown rust developed when infection was obtained on *Lepargyrea canadensis of* the family Eleagnaceae through inoculation with teliospores from *Calamagrostis purpurascens*. It is not yet clearly understood just what alternate-host range and particular specialization that form of crown rust may have which produces abundant aecia on *Rhamnus purshiana*, a species indigenous to British Columbia, California, Oregon, Washington, and Idaho. The data here presented show the alternate-host specialization and the marked development of resistance and susceptibility of the physiologic forms Avenae and Calamagrostidis. These, combined with the extension of the alternate-host range beyond the genus Rhamnus and, in fact, outside the family Rhamnaceae, allow no alternative but to consider the crown-rust organism as one species with a wide alternate-host range. Crown rust, then, must remain as *Puccinia coronata* Corda.

Another point of much importance in connection with the rust reaction of Rhamnus is the influence exerted by different species of the host on the stability of the physiologic forms of the rust.

Among the European students of crown rust, Treboux alone, as noted earlier, presented results at variance with those of other workers. He obtained infection of grasses directly through aeciospores, while most of the other European investigators have sought to infect Rhamnus through teliospores of various grasses. Treboux (*12*, *13*) produced urediniospores on *Avena sativa* through inoculation with aeciospores from *R. frangula*. He also found that the aeciospores from *R. cathartica* infected *Agrostis stolonifera*, *Calamagrostis arundinacea*, and *Phalaris arundinacea*, all of which are listed as hosts of *Puccinia coronata* by Klebahn and Mühlethaler. On the other hand, in localities where only *R. frangula* occurs, two of the hosts of *P. coronifera*, namely, *A. alba* and *Poa pratensis*, were infected with *P. coronata*. Treboux took this to mean that specialization as reported by Klebahn and others probably did not exist. However, to the writer, Treboux's results, in connection with data here presented, suggest that the stability of the physiologic forms was changed through their culture on the Rhamnus hosts.

As shown in Table 5, teliospores from *Avena sativa* produced infection on eight species of Rhamnus. The aeciospores from each of these, in turn, caused abundant infection on *Notholcus lanatus* and *A. sativa*. This result is directly opposed to ordinary experience, for the form of crown rust on *N. lanatus* is highly specialized (*9*), only occasionally producing a subnormal infection on *A. sativa* when urediniospores are used as inoculum. Again, teliospores from *Calamagrostis canadensis* caused infection on *R. lanceolata*, these aeciospores producing urediniospores on *A. sativa* many times during a period of three years. It has been shown in an earlier paper (*9*) that the urediniospores on *C. canadensis* can only occasionally produce a subnormal infection on *A. sativa* and vice versa. On the other hand, teliospores from *A. sativa* produced aeciospores on *R. lanceolata* which produced urediniospores on *C. canadensis* (Table 5). This suggests that the physiologic-form relationship as expressed by the gramineous hosts may be altered by the Rhamnus host.

SUMMARY

The division of *Puccinia coronata* Corda into two species, as has been done by Klebahn, in Europe, is not justified in America.

The fact that *Rhamnus dahurica* produces aecia through teliospores from *Avena sativa* prevents this species of Rhamnus from functioning as a differential host for *Puccinia coronifera* Kleb. and *P. himalensis* (Barcl.) Diet., although Eriksson and Mühlethaler in Europe have claimed that it could so serve.

The alternate hosts of *Puccinia coronata* are not restricted to the genus Rhamnus or even to the family Rhamnaceae.

Thirteen species and one variety of Rhamnus, indigenous to the United States, and three species native to Europe, were investigated as possible alternate hosts of crown rust, from 1 to 28 times each, making a total of 364 trials. When *Rhamnus californica* var. *tomentella*, *R. caroliniana*, *R. cathartica*, *R. ilicifolia*, *R. lanceolata*, *R. pachyphylla*, *R. pinetorum*, *R. rubra*, and *R. smithii* were inoculated with teliospores from *Avena sativa*, the resulting infection was normal in at least one trial. *R. alnifolia*, *R. californica*, *R. crocea*, *R. dahurica*, and *R. tinctoria* also are susceptible, and may function

as alternate hosts of the Avenae form of *P. coronata*. *Rhamnus frangula*, *R. nevadensis*, and *R. purshiana* reacted with marked resistance to the physiologic form on *Avena sativa*.

Rhamnus alnifolia, *R. californica*, *R. crocea*, *R. lanceolata*, and *R. smithii* developed normal infection when inoculated with teliospores from *Calamagrostis canadensis*. It also was found that *R. californica* var. *tomentella*, *R. cathartica*, *R. pinetorum*, and *R. rubra* may function as alternate hosts of this physiologic form. The other eight species showed marked resistance.

Teliospores from *Festuca elatior* caused normal infection on *Rhamnus alnifolia*, *R. californica*, *R. cathartica*, and *R. lanceolata*. *R. californica* var. *tomentella*, *R. caroliniana*, *R. crocea*, *R. rubra*, and *R. smithii* produced a few aecia. Twelve trials on the seven remaining species showed that *R. ilicifolia* produced pycnia and *R. pachyphylla* developed flecks only.

Teliospores from *Notholcus lanatus* produced pycnia on *Rhamnus lanceolata*.

Ceanothus americanus, *Lepargyrea argentea*, and *Zizyphus lycioides* did not respond as alternate hosts of crown rust. *Berchemia scandens* was infected with teliospores from *Avena sativa*. *Lepargyrea canadensis* produced aecia when exposed to infection with teliospores from *Calamagrostis purpurascens*.

Rhamnus species, functioning as alternate hosts, may alter the physiologic response of specialized forms.

A total of 1,021 plantings of *Rhamnus cathartica* were found in the upper Mississippi Valley. Of these, 282 were rural and 739 urban, with a total of 93,041 bushes, besides 44,395 feet of buckthorn hedge of uncounted bushes. In addition, many areas of escaped plants were located in Illinois, Montana, and Wisconsin.

During the past nine years in Iowa, aecial infection has appeared on *Rhamnus cathartica* and *R. lanceolata* previous to the development of uredinia on *Avena sativa*. These two species of Rhamnus have been instrumental in starting local and general epidemics in Iowa within the past two years.

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THE INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON THE DEVELOPMENT OF YELLOWS IN CABBAGE SEEDLINGS[1]

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INTRODUCTION

The effect of temperature upon the development of the disease of cabbage caused by the vascular parasite *Fusarium conglutinans* Wollenw. has been studied by Gilman (*5*)[2] and Tisdale (*14*). Gilman found that this disease appears in its most destructive form in the fields under conditions of hot, dry weather, whereas it develops but slightly during moist, cool weather. Tisdale determined more exactly the relation of soil temperature to the development of the disease in cabbage seedlings. He found that cabbage yellows develops in seedlings growing in "sick" soil at soil temperatures ranging from 17° to 35° C., the optimum for rapid development of the disease being from 26° to 29°. This optimum temperature coincides with the optimum for vegetative growth of the fungus in pure culture and is distinctly above the optimum for growth of cabbage seddlings, which Tisdale found to be about 20°. He found also that yellows occurs at any percentage of soil moisture at which the cabbage seedlings grow, but that it develops most rapidly and destructively at about 19 per cent of the dry weight. This soil moisture is also the most favorable for the normal growth of the host plant. Tisdale found that seedlings of the resistant Wisconsin Hollander and the susceptible Commercial Hollander strains up to the age of 20 days were almost equally susceptible to yellows. However, as the age of the plants increased resistance became more pronounced in the resistant strain. He used only plants of Wisconsin Hollander and Commercial Hollander.

The purpose of the study herein reported was sixfold: (1) To determine the range of soil temperatures at which yellows occurs in the young seedlings of Wisconsin All Seasons and Commercial All Seasons strains; (2) to determine the effect of various soil temperatures upon the resistant quality of the selected cabbage strains; (3) to study further the range of soil temperatures for the development of yellows in both resistant and susceptible cabbage strains; (4) to determine the difference in the development of the disease in cabbage plants grown at high and at low temperatures before being transplanted to diseased soil; (5) to study the effect of different combinations of air and soil temperatures on yellows development; and (6) to study further the influence of soil moisture upon the occurrence of yellows.

[1] Received for publication Apr. 1, 1926; issued December, 1926. This, the second of two articles relating to the yellows disease of cabbage (*13*)[2], was presented to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of doctor of philosophy. Grateful acknowledgment is made to L. R. Jones, of the University of Wisconsin, and J. C. Walker, of the Office of Vegetable and Forage Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for suggestions and helpful criticisms throughout the progress of the work.

[2] Reference is made by number (italic) to "Literature cited," p. 991.

METHODS AND EQUIPMENT

The apparatus employed for controlling the soil temperature in these experiments was what is known as the Wisconsin soil-temperature tank. Since this apparatus has been described by Jones (*9*) and a modified form by Dickson (*3*), further description is unnecessary. The temperatures below 20° C. were regulated by running in small quantities of cold water as often as required to keep the temperature of the water in the tank fairly constant. The higher temperatures were maintained by the use of electric lamps controlled by thermostats. With careful attention at regular intervals, the temperatures seldom varied more than 1° or 2° from those desired, and then for only a short time.

Most of the experiments were conducted in a greenhouse room with the temperature at about 20° C. This was fairly constant except during the spring months beginning late in March, when the sun caused a rise in temperature which could not be entirely eliminated by means of the blower. Other experiments were conducted under different air temperatures, which will be discussed separately with the various experiments.

RECEPTACLES

The culture cans were made of galvanized sheet iron, cylindrical, about 6 inches in diameter and 10 inches deep. They were prepared for use by first being brought to an even weight by the addition of coarse gravel to the lighter cans. A 2-inch clay pot with a one-half-inch hole in the bottom was upturned in the bottom of the can and the soil put in up to within about 1½ inches of the top, an equal weight of soil being placed in each can. The water was supplied through a test tube with a broken bottom, which was placed in the center of the receptacle with the lower end connected with the hole in the bottom of the flowerpot. After the cans were filled with soil they were so placed in the tanks that the surface of the soil was about level with the water in the tank.

SOIL

The naturally infested soil used in all the experiments was obtained from a "cabbage sick" field in Kenosha County, Wis. Before being used, the soil was screened and uniformly mixed with a virgin clay loam in the ratio of 5 parts of the infested soil to 1 of virgin soil.

The moisture content of the soil in all the temperature experiments, with the exceptions mentioned later, was kept at about 20 per cent of the dry weight or 40 per cent of the moisture-holding capacity, as determined by the 10-inch soil cylinder method. Tisdale (*14*) found that with favorable temperature the yellows disease develops most rapidly and destructively in soil with about 19 per cent moisture on the dry-weight basis, or 40 per cent on the wet basis. This moisture content was used in order to give the plants as severe a test as possible. The receptacles were weighed at regular intervals and water added to keep the soil at about the moisture content desired.

SEED

The Wisconsin All Seasons seed used in all the experiments unless otherwise specified was from a lot of seed grown in the Puget Sound section of Washington in 1921. This strain of cabbage had been

proved by Jones et al. (*10*) to be highly resistant to yellows under field conditions. The Commercial All Seasons seed was taken from a single lot grown on Long Island, N. Y., in 1921 and obtained from a seed company in Madison, Wis. Two lots of Wisconsin Hollander seed were also used. Lot 1 was grown in 1921 by W. A. Walker at Racine, Wis., and lot 2 was grown in Washington State in 1923 from the seed of lot 1. The Commercial Hollander seed was grown in Denmark in 1921.

RELATIVE SUSCEPTIBILITY TO YELLOWS OF "RESISTANT" AND "SUSCEPTIBLE" STRAINS OF CABBAGE SEEDLINGS

Seed of Wisconsin All Seasons and Commercial All Seasons strains were planted in naturally infested soil in the receptacles previously described and placed in tanks at temperatures of 16°, 20°, 24°, 28°, and 32° C., four cans of each variety at each temperature. When the seedlings had emerged from the soil they were thinned so as to leave 10 plants to each receptacle.

Data were taken 10, 13, 17, and 20 days after planting. Percentages of yellows, as given in Table 1, include the plants which showed the disease in incipient stages as well as those which died of it. The All Seasons strains tested reacted to yellows in a way similar to those tested by Tisdale, who found that there was little difference in percentage of disease between resistant and susceptible Hollander strains up to the age of 29 days. However, the disease was somewhat slower in developing in the plants of the resistant strains, and the final percentages of disease were somewhat lower in these than in plants of the susceptible strain.

TABLE 1.—*Percentage of cabbage seedlings growing in naturally infested soil which developed yellows at different soil temperatures*

[Air temperature from 20° to 22° C.]

Soil temperature °C.	Number of plants of each variety	Percentage of Commercial All Seasons diseased after—				Percentage of Wisconsin All Seasons diseased after—			
		10 days	13 days	17 days	20 days	10 days	13 days	17 days	20 days
16	40	0	0	0	0	0	0	0	0
20	40	0	0	12	32	0	0	15	22
24	40	8	42	54	74	8	35	50	53
28	40	25	60	65	82	10	32	45	80
32	40	22	62	76	70	20	38	50	55

It has been observed by Edgerton and Moreland (*4*) that tomato wilt develops more slowly in a resistant strain and that the plants do not die as rapidly as those of the susceptible strains. According to these investigators—

> all varieties, even the most resistant, when grown under the most favorable conditions, show a rather heavy infection with the wilt disease. Susceptible varieties, however, take the wilt earlier, die quicker, and in most cases show a higher percentage disease than resistant ones.

Somewhat the same condition seems to obtain in the strains of cabbage so far produced, except one recently developed by Walker et al. (*17*), which appears to be practically immune to yellows. Under field conditions there may be a high percentage of yellows in

some of the resistant strains, but most of these are able to withstand or resist the attack of the fungus to a greater degree than the susceptible strains. The difference between the resistant and susceptible strains seems to lie in the ability of the former to prevent the development of the fungus after it has once gained access to the tissues. There is evidently a sort of balance reached between the host and parasite in the case of the resistant strains under ordinary field conditions. When the soil temperature goes up and the soil moisture is reduced, this balance is upset and the fungus makes further inroads on the tissues of the plant. If these conditions prevail for a sufficient length of time the plants may be killed; but in general they do not persist long enough to cause the death of many plants of the resistant strains. With the advent of cooler weather the earlier balance between the host and the parasite is restored and the cabbage plants again become "resistant" and develop normally.

In January, 1923, plants of the Wisconsin Hollander and Commercial Hollander strains, grown in virgin soil for 55 days at an air temperature of from 14° to 18° C., were transplanted to receptacles containing naturally infested soil. Four plants of each sort were placed in separate receptacles, one can of each kind in each of five tanks maintained at temperatures of 12°, 18°, 24°, 28°, and 32°, respectively. Observations were made 13, 21, and 33 days after transplanting. The results, shown in Table 2, indicate that within 13 days after they were placed in diseased soil at temperatures of 24°, 28°, and 32°, half or more of the plants of the resistant Wisconsin Hollander showed symptoms of disease. Two of this strain in the 24° tank were diseased at the end of the thirteenth day after transplanting, and at the end of 33 days all four were diseased and two were dead. In the case of the susceptible Commercial Hollander, however, three-fourths of the plants in the 24° tank were dead by the thirteenth day and all were dead by the twenty-first day. At the conclusion of the experiment it was noted that all of the susceptible strain were dead in the 24°, 28°, and 32° tanks, whereas 50 per cent of the resistant strain were still alive in the 24° tank, 75 per cent in the 28° tank, and 75 per cent in the 32° tank.

TABLE 2.—*Variations in susceptibility among cabbage seedlings of Commercial Hollander and Wisconsin Hollander strains, transplanted from healthy to naturally infested soil, and grown at different soil temperatures*

Soil temperature (°C.)	Number of plants tested	Commercial Hollander					Wisconsin Hollander				
		Number of plants diseased after—			Per cent diseased at end of experiment	Per cent dead at end of experiment	Number of plants diseased after—			Per cent diseased at end of experiment	Per cent dead at end of experiment
		13 days	21 days	33 days			13 days	21 days	33 days		
12	4	0	0	(a)	0	0	0	0	(a)	0	0
18	4	0	0	(a)	0	0	0	0	(a)	0	0
24	4	3	4	4	100	100	2	4	[b] 4	100	50
28	4	3	4	4	100	100	3	3	3	75	25
32	4	3	4	4	100	100	2	3	3	75	25

[a] Plants in diseased soil at 12° and 18° C., were removed 21 days after transplanting to tank at 28°. The Commercial Hollander plants were all dead within 10 days, and 6 of the 8 Wisconsin Hollander plants were diseased at the end of 24 days.

[b] One plant showing severe yellows symptoms put out new roots near the surface of the soil and survived the attack of the fungus.

In Table 3 are summarized the data from several soil temperature experiments with plants grown under different conditions. The results are rather difficult to interpret because of the great variation in the percentage of disease among plants of the different lots These data certainly confirm some things, however, that have been noted before, namely, that there is a wide variation in the percentage of disease, its severity, and the length of the incubation period among plants of both resistant and susceptible strains. The slight differences in the size and vigor of the plants before they were placed in diseased soil might have been caused by a difference in environmental factors such as wate · supply and sunlight, or by rapidity of growth, and this initial strength or weakness might account for some variations in susceptibility among the seedlings to the yellows disease. In addition to these factors, there must also be the factor of heterozygosity in the genetic composition of the plants. Unless resistance is inherited as a simple Mendelian character it is quite likely that the average lot of cabbage seed is heterozygous for the factors for resistance, since the cabbage plant is normally cross fertilized. Furthermore, in the selection of resistant strains there has been no attempt to acquire homozygosity by selfing of plants through a series of generations, simple mass selection having been made in all cases until the very recent work of Walker (*16*).

In general, it seems that vigorous plants are most subject to the attack of the yellows parasite. In some cases it was found that plants of the commercial strains, which are usually highly susceptible to yellows, when grown under conditions of low soil moisture and high soil temperature, during periods of low light intensity, often remained in diseased soil for from two to four weeks without showing a very high percentage of disease. These plants grew very slowly and the leaves became somewhat leathery in texture. Especially was this noticeable during midwinter when there was often no direct sunlight for several days in succession.

In the next experiment, which was begun on February 8 and ended March 9, seven soil temperatures were used beginning at 15° C., and increasing in increments of 3° to 33°. Wisconsin All Seasons and Commercial All Seasons plants were grown in healthy soil at an air temperature of about 20° for 55 days before being removed to diseased soil. Three plants were placed in each receptacle, and five receptacles containing resistant plants and three containing susceptible plants were used. The receptacles were kept at 20° air temperature for 24 hours before they were placed in the tanks.

Beginning 9 days after transplanting, observations were made at intervals until the plants had been in diseased soil for 30 days. The results are shown in Table 4 and Figures 1, 2, and 3. It will be noted from an examination of Figure 2 that beginning at a soil temperature of 24° C. and continuing to 33° there is very little difference in resistance between the plants of the resistant strain and those of the susceptible. Even less disease occurred in the latter strain at 33° than in the resistant strain, no disease being evident in any of the 9 plants of the commercial strain, whereas 3 of the 15 plants of the resistant strain showed yellows, 2 of them having succumbed to the disease. At 21°, however, there was a striking difference in the percentage of diseased plants in the resistant and susceptible strains,

33 per cent showing disease in the Wisconsin All Seasons at this temperature as compared with 89 per cent in the Commercial All Seasons plants.

TABLE 3.—*Effect of high soil temperature and low soil moisture upon the development of yellows in cabbage plants—summary of four experiments*

Variety	Date of transplanting	Age of plants in days	Soil temperature before transplanting	Air temperature	Soil temperature after transplanting	Number of days in sick soil	Number of plants	Per cent diseased	Per cent dead	Remarks
			°C.	°C.	°C.					
Wisconsin: All Seasons.	Jan. 22, 1923	60	12–15	25–28	20–23	60	50	* 27	20	In flats.
Wisconsin: Hollander.	Jan. 26, 1923	55	14–16	20–23	24	23	4	100	75	In tanks.
					28	23	4	100	100	
					32	23	4	100	50	
Commercial Hollander.	.....do.....	55	14–16	20–23	24	23	12	100	80	Do.
Wisconsin All Seasons.	Mar. 9, 1923	82	16–18	28	28	45	20	57	46	Do.
Commercial All Seasons.	.....do.....	82	16–18	28	28	45	10	90	90	Do.
Wisconsin All Seasons.	Apr. 4, 1923	90	15–22	28	28	23	36	15	11	Do.
Wisconsin All Seasons.	Apr. 28, 1923	60	20–22	24	24	33	48	50	32	Do.
Commercial All Seasons.	.....do.....	60	20–22	24	24	33	16	100	100	Do.

* Plants in flats of diseased soil where soil temperature and soil moisture were not under control

TABLE 4.—*Effect of temperature on the development of yellows in Wisconsin All Seasons and Commercial All Seasons cabbage plants*

WISCONSIN ALL SEASONS

Soil temperature (°C.)	Number of plants of each variety	Percentage of plants diseased or dead after—															
		9 days		12 days		15 days		18 days		21 days		24 days		27 days		30 days	
		Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead
15	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	* 7	0
21	15	0	0	0	0	0	0	0	0	7	0	13	0	33	0	33	0
24	15	0	0	6	0	13	0	27	0	40	0	47	0	59	27	66	27
27	15	7	0	27	0	33	0	46	6	60	20	70	52	70	70	77	70
30	15	0	0	13	0	27	0	52	7	60	40	67	52	70	59	70	59
33	15	0	0	13	0	13	0	13	0	20	0	20	0	20	13	20	13

COMMERCIAL ALL SEASONS

15	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	9	0	0	0	0	0	0	0	0	0	0	0	0	11	0	22	0
21	9	11	0	22	0	22	0	22	0	33	0	44	0	78	33	89	33
24	9	11	0	33	0	44	0	67	11	67	22	67	67	67	67	67	67
27	9	11	0	22	0	22	0	33	11	78	33	78	44	78	55	78	55
30	9	11	0	33	0	33	0	44	22	78	22	89	66	89	66	89	66
33	9	0	0	0	0	0	0	0	0	11	0	11	0	11	0	11	0

* One plant slightly diseased.

The curves for plants killed by yellows, shown in Figure 1, parallel those for percentage of disease quite closely up to 24° C., but a difference in percentage of plants killed by yellows at 24° is observed

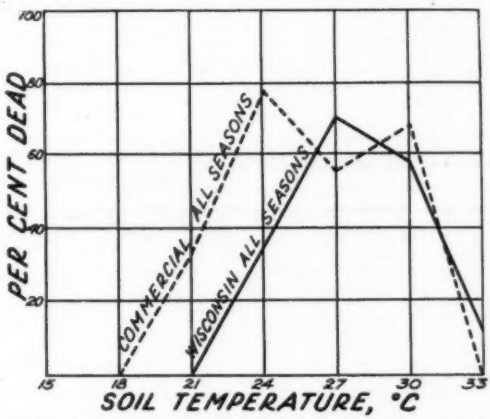

FIG. 71.—Comparison of the death rate from yellows of Commercial All Seasons and Wisconsin All Seasons plants grown for 30 days in naturally infested soil. (See Table 4)

comparable to the percentage of disease shown in Figure 2 at 21°. Although at 27° a higher percentage of plants of the resistant Wisconsin strain were killed than of the more susceptible commercial strain, the curve for death of the latter has two maxima, one at 24°

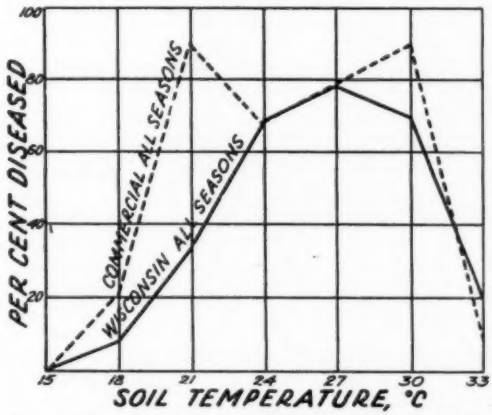

FIG. 2.—Comparison of the development of yellows in Commercial All Seasons and Wisconsin All Seasons plants grown 30 days in naturally infested soil. (See Table 4)

and the other at 30°. These results differ from those obtained by Tisdale (*14*), who found 29° to be the optimum temperature for yellows development in the Wisconsin Hollander variety and 26° for plants of the Commercial Hollander. The curve would probably be straightened somewhat if the average of several experiments was used.

The incubation period of the disease varied a good deal in the two strains with the temperature of the soil. For example, as shown in Figure 3, B, at a soil temperature of 21° the maximum percentage of disease was reached in the Wisconsin All Seasons plants 27 days after transplantation to diseased soil, whereas the maximum among the susceptible plants was not reached until the thirtieth day. The highest percentage of disease at 24° developed in the susceptible strain on the eighteenth day, but in the resistant strain the highest percentage was not reached until the thirtieth day, although the same percentage of disease developed in both strains. In Figure 3, A, it is shown that a higher percentage of plants were killed in the resistant strain at 27° than in the commercial strain. However, at 24° the reverse was true, although the same amount of disease was present in both strains at this temperature. It has been noted throughout the course of these investigations that, although there may be a high percentage of disease among the plants of the resistant strains, the percentage killed is usually much smaller than in the commercial strains. And this is more striking at 21° and 24°, which represent most nearly the extreme of the mean soil temperature ordinarily encountered in the field.

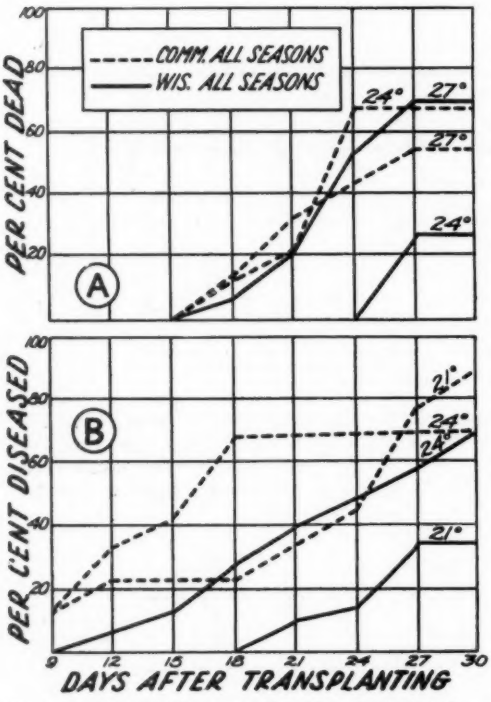

FIG. 3.—Comparison of the rate at which yellows developed in Commercial All Seasons and Wisconsin All Seasons plants grown in naturally infested soil at different temperatures. (See Table 4)

RELATION OF SOIL AND AIR TEMPERATURES TO THE DEVELOPMENT OF YELLOWS IN CABBAGE SEEDLINGS

Clayton (*1*), in his study of the effect of soil temperature on the development of tomato wilt, found that the optimum temperature for growth of the disease was about 29° C. Edgerton and Moreland (*4*) found that tomato wilt developed more slowly if the temperature was much below 29°. Clayton (*2*) grew tomato plants in soil at temperatures of 17°, 27°, and 33° at each of three air temperatures, namely, 17°, 27°, and 33°, and found that of the nine combinations

of air and soil temperatures only two produced wilt to any appreciable extent. These were (1) soil and air temperature both 27°, and (2) soil 27° and air 33°. When the plants were kept at a high soil temperature and a comparatively low air temperature the vascular bundles of the plants became discolored from the ground line downward, showing that the parasite had penetrated the lower parts of the plant, but no symptoms of wilt appeared above the ground line. Plants from a soil and air temperature of 33° showed only very faint traces of wilt and slight discoloration of the vascular bundles. These results seem to indicate that the tomato-wilt organism is unable to penetrate to any great extent those parts of the plant which are surrounded by cool air, that is, air at about 17°.

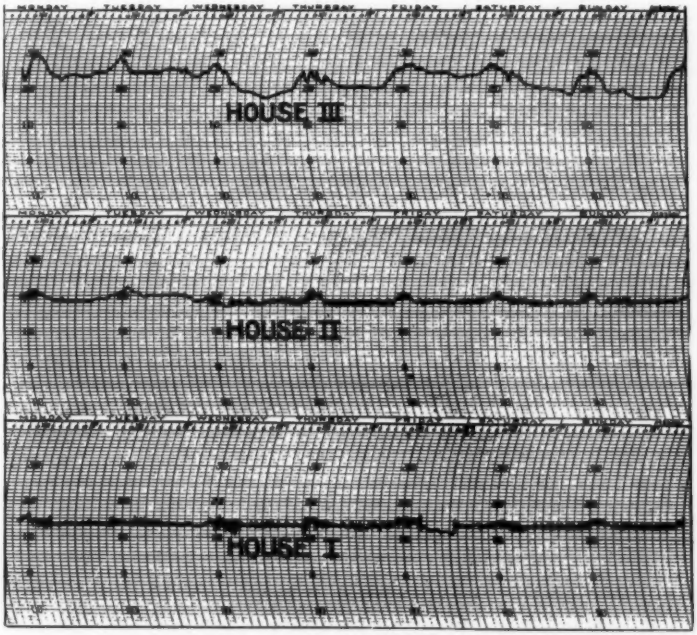

FIG. 4.—A weekly record of air temperatures kept in the course of the experiment showing the effect of different air temperatures on the development of cabbage yellows

With these results in mind, the following experiment was conducted with cabbage plants of Wisconsin Hollander and Commercial Hollander strains 77 days old. Lot 1 consisted of plants of both strains grown in healthy soil at air and soil temperatures of from 12° to 16° C., and lot 2 of plants grown at 25° to 28° air and soil temperature. Both lots were transferred to receptacles containing diseased soil as previously described.

The temperature of the air in the three greenhouse rooms was controlled to within fairly narrow limits except in the high-temperature house, where it could not be kept under perfect control during a season of exceptionally cold weather. Hourly temperature records were kept in three houses. Figure 4 shows a representative weekly

record for each of the three houses, and Figure 5 represents the mean daily temperature for each house during the 30 days of the experiment. It was planned to keep the high-temperature house at 28°, but the excessively cold weather brought the mean average down to 25°; the temperatures of the other houses were maintained at a mean average of 19° and 14.5°, respectively.

The results of this experiment are given in Table 5 and Figure 6. The disease developed more rapidly and in most cases was more severe in lot 1, plants grown at 12° to 16° C. before transplantation, than in lot 2, plants grown at 25° to 28° before transplantation. This is brought out strikingly in Figure 6, A, the plants in *b* (lot 2) being only slightly diseased while those in *a* (lot 1) were severely attacked, two of them having succumbed. This would seem to

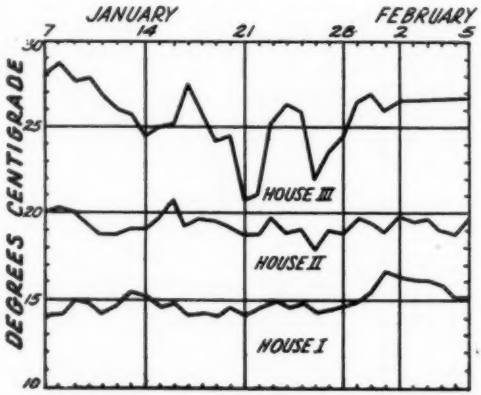

FIG. 5.—Mean daily air temperature in each of the three greenhouse rooms used during the experiment dealing with the effect of air temperature on the development of cabbage yellows

indicate that plants grown at higher temperatures before being transplanted to diseased soil are more resistant than those grown at lower temperatures.

Harvey (*6*), in his studies of the hardening process in plants, found that cabbage plants hardened for five days at 3° C. were rich in reducing sugars as glucose, and disaccharides as sucrose when compared with nonhardened plants grown at 18° to 25°, which contained a higher percentage of polysaccharides as starch. The hardened plants contained 7.64 per cent carbohydrates as compared with 5.16 per cent in the nonhardened plants. Lidforss (*11*) found this predominance of monosaccharides and disaccharides to be a common transformation in plants during the cold seasons. Hasselbring and Hawkins (*7*) found a similar condition in sweet potatoes kept at low temperatures. Rosa (*12*) found that the pentosan content of hardened cabbage plants is distinctly greater than that of nonhardened plants. Both of these investigators concluded that there is a slightly lower percentage of moisture in hardened cabbage plants than in plants grown at the higher temperatures, and Harvey (*6*) indicates that in the hardened plants there is an increase in total nitrogen and phosphorus.

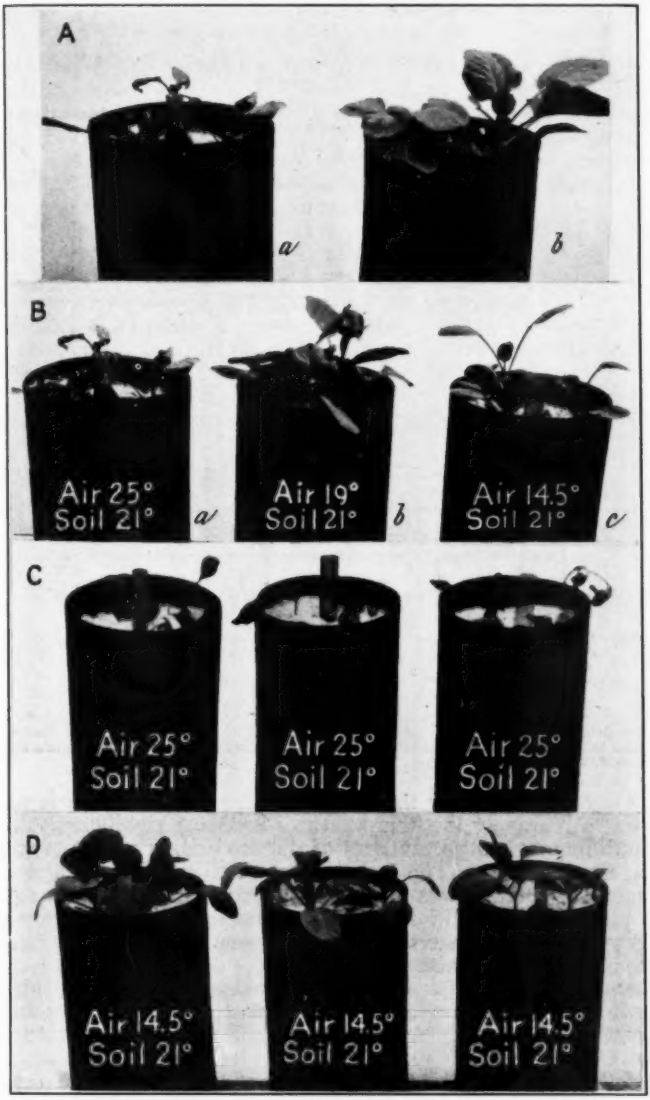

FIG. 6

A.—Commercial Hollander plants grown in healthy soil for 77 days before transplantation to naturally infested soil in tanks, at soil temperature of 21° C. and air temperature of 28°: *a*, Plants grown at 12° to 16° before transplanting; *b*, plants grown at 25° to 28° before transplanting. Note in *a* that all three plants are diseased and two are dead; in *b* two are very slightly diseased

B.—Commercial Hollander plants kept in diseased soil for 30 days at a soil temperature of 21° C.: *a*, At air temperature of 25° all plants dead; *b*, at air temperature of 19° one plant diseased, the others healthy; *c*, at air temperature of 14.5° no disease evident

C.—Wisconsin Hollander plants kept in diseased soil for 30 days at a soil temperature of 21° C. Of 9 plants kept at a daily average air temperature of 25°, 7 dead, 2 severely diseased

D.—Wisconsin Hollander plants kept at a daily average air temperature of 14.5°, all plants healthy

TABLE 5.—*Effect of different soil and air temperatures upon the development of cabbage yellows*

[Plants of lot 1 were grown at 12° to 16° C. for 55 days before transplantation to diseased soil; those of lot 2 were grown for the same length of time at 25° to 28°]

WISCONSIN HOLLANDER

Air temperature (° C.)	Soil temperature (° C.)	Lot 1					Lot 2				
		Total number of plants	Per cent diseased after 18 days	Per cent diseased after 24 days	Per cent diseased after 30 days	Per cent dead after 30 days	Total number of plants	Per cent diseased after 18 days	Per cent diseased after 24 days	Per cent diseased after 30 days	Per cent dead after 30 days
14.5	15	9	0	0	0	0	9	0	0	0	0
	21	9	0	22	[a] 22	0	8	0	0	0	0
	27	8	12	37	37	25	7	0	12	37	12
19	15	9	0	0	0	0	9	0	0	0	0
	21	8	12	24	60	37	9	0	0	0	0
	27	8	24	36	72	48	8	12	36	60	24
25	15	9	0	0	0	0	9	0	0	0	0
	21	8	24	48	74	36	8	0	12	24	12
	27	8	48	72	84	60	8	12	36	48	36

COMMERCIAL HOLLANDER

14.5	15	3	0	0	0	0	3	0	0	0	0
	21	3	0	0	0	0	3	0	0	0	0
	27	3	33	66	100	100	3	33	66	100	100
19	15	3	0	0	0	0	3	0	0	0	0
	21	3	0	33	66	66	3	0	0	33	0
	27	3	66	100	100	100	3	33	66	100	100
25	15	3	0	0	0	0	3	0	0	0	0
	21	3	100	100	100	100	3	0	33	66	66
	27	3	100	100	100	100	3	33	66	100	100

[a] Two plants in this lot showed only slight yellowing with no dwarfing, distortion, or shedding of leaves as is typical of the disease at the higher temperatures, but the fungus was found in the main stem and the petioles of the older leaves.

It is uncertain whether the changes taking place in cabbage plants exposed to relatively low temperatures bear any significant relation to the differences in percentages of disease among plants grown at higher temperatures. The temperature at which Harvey and Rose hardened their plants was much lower than that used in the experiment just described, 12° to 16° C. However, it seems likely that changes of the same general nature may occur in plants when grown at somewhat higher temperatures.

When the temperature of the soil was kept constant the rapidity with which yellows developed varied greatly with changes in the temperature of the air. At the highest soil and air temperature all of the Commercial Hollander plants of lot 1 were dead after 12 days, followed soon by lot 2 and by lot 1 of the Wisconsin Hollander plants, which were all diseased except 1 after 15 days. In the 19° house the incubation period of the disease lasted several days longer than in the 25° house, but was about the same as in the 14.5° house.

The effect of varying the air temperature while the soil temperature remains constant is well illustrated by Figure 6, B. Here the three receptacles were kept for 30 days at a uniform soil temperature of 21°. The plants in *a* were held at an average air temperature of 25°, those in *b* at 19°, and those in *c* at 14.5°. At the end of the 30 days all of the plants in *a* were dead; of those in *b* one was slightly diseased and the others were healthy; in *c* no disease appeared.

Figure 6, C and D, shows even more strikingly the influence of air temperature on yellows development. Both lots of the Wisconsin Hollander plants shown in this figure were kept at a soil temperature of 21° C., but the plants in C were kept at an average air temperature of 25°, whereas those in D were kept at an average air temperature of 14.5°. In C all of the nine plants were dead or diseased, but in D the plants showed no symptoms of disease.

No disease symptoms were evident in the Commercial Hollander plants in either lot 1 or lot 2 in the 21° tank in the 14.5° house. But upon examination it was found that the vascular bundles of these plants were discolored and contained the mycelium of the yellows fungus up to 2 inches above the soil line. The following experiment was conducted to ascertain whether plants of the susceptible strains

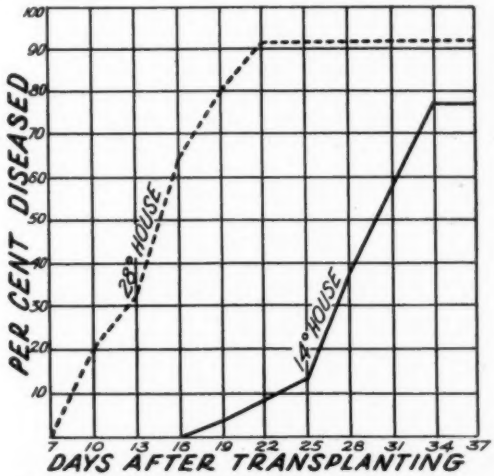

FIG. 7.—Comparison of the rate of development of yellows in two lots of Commercial Hollander cabbage seedlings kept at a constant soil temperature of 21° C. and air temperatures of 14° and 28°, respectively. (See Table 6)

would consistently exhibit this lack of typical yellows symptoms when grown at high soil temperature and low air temperature.

In each of 16 receptacles containing diseased soil were placed four plants of the Commercial Hollander strain 40 days old. Eight of these receptacles were then put in the 21° tanks of the 28° house and eight in the 21° tanks of the 14° house. In the 28° house yellows appeared on the eighth day, and by the twenty-second day 92 per cent of the plants were diseased; in the 14° house the first symptoms of disease appeared on the eighteenth day, after which the disease progressed slowly until the thiry-fourth day. The results of this experiment are shown in Table 6 and Figures 7 and 8. The difference in percentage of plants killed and the rate at which they were killed is pronounced. The death curve (fig. 8) parallels the disease curve (fig. 7) quite closely in the 28° house, but in the 14° house there is little similarity between the two. In this house the first plant was not killed until the twenty-sixth day, and after 37 days 72 per cent of the original number still survived.

TABLE 6.—*Variations in susceptibility between two lots of Commercial Hollander cabbage seedlings grown at a soil temperature of 21° C. and air temperatures of 28° and 14° respectively*

PER CENT DISEASED

Air temperature (° C.)	Number of plants	After 7 days	After 10 days	After 13 days	After 16 days	After 19 days	After 22 days	After 25 days	After 28 days	After 31 days	After 34 days	After 37 days
28	32	0	20	32	64	80	92	92	92	92	92	92
14	32	0	0	0	0	4	8	12	36	56	76	76

PER CENT DEAD

28	32	0	4	10	30	52	68	80	80	80	80	80
14	32	0	0	0	0	0	0	0	4	4	16	28

In all of the experiments conducted by the writer no case was found where the yellows disease developed at a soil temperature as low as 15° C. Since the yellows fungus is found in the roots of many plants grown at a soil temperature of 15°, it was believed that if the

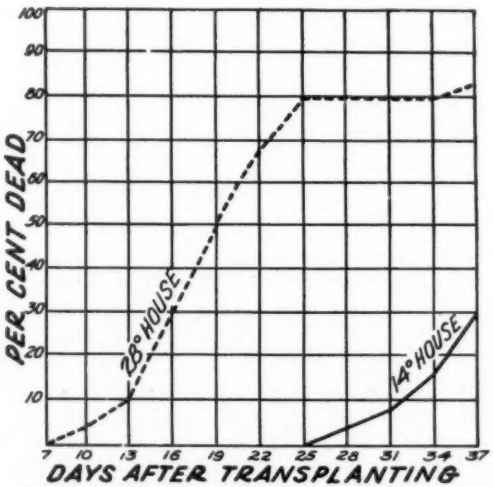

FIG. 8.—Comparison of the death rate in two lots of Commercial Hollander cabbage seedlings kept at a constant soil temperature of 21° C. and air temperature of 14° and 28°, respectively. (See Table 6)

plants were grown at a high air and low soil temperature the fungus might penetrate the parts of the plants exposed to the warm air, but experiment proved that such was not the case. The fungus was found to be present in the tap roots of many of the plants tested but never above the soil line in the stems.

In the experiment next to be described the soil moisture was kept at about 27 per cent of the dry weight, or 60 per cent of the moisture-holding capacity. Wisconsin Hollander and Commercial Hollander plants grown in healthy soil for 62 days at 20° to 23° C. were trans-

planted to receptacles containing naturally infested soil, two plants to each can. These plants were healthy in appearance and about the size of those commonly used for transplanting to the field. The plants were kept for 30 days at soil temperatures ranging from 15° to 33° in increments of 3°.

TABLE 7.—*Comparative resistance to Fusarium conglutinans of Wisconsin Hollander and Commercial Hollander cabbage plants grown in naturally infested soil at 60 per cent moisture-holding capacity for 30 days*

WISCONSIN HOLLANDER

Soil temperature (°C.)	Number of plants	Per cent diseased											
		After 12 days		After 15 days		After 21 days		After 24 days		After 27 days		After 30 days	
		Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead
15	10	0	0	0	0	0	0	0	0	0	0	0	0
18	10	0	0	0	0	0	0	0	0	0	0	0	0
21	10	0	0	0	0	0	0	0	0	0	0	0	0
24	10	0	0	0	0	0	0	0	0	0	0	10	0
27	10	0	0	0	0	0	0	0	0	0	0	10	0
30	10	0	0	20	0	30	0	30	0	40	10	50	30
33	10	0	0	0	0	0	0	0	0	0	0	10	0

COMMERCIAL HOLLANDER

15	6	0	0	0	0	0	0	0	0	0	0	0	0
18	6	0	0	0	0	0	0	0	0	0	0	0	0
21	6	0	0	0	0	0	0	0	0	33	0	50	0
24	6	17	0	33	0	83	17	83	33	100	50	100	83
27	6	0	0	33	0	50	0	50	33	100	50	100	100
30	6	33	0	100	0	100	66	100	100	100	100	100	100
33	6	17	0	17	0	17	0	33	0	33	17	67	33

The results of this experiment, given in Table 7 and Figure 9, are strikingly different from those in most of the preceding work. Here for the first time are conditions in which the differences in resistance between the plants of the so-called "resistant" and "susceptible" strains approximate those in the field. A much smaller percentage of disease was present in the plants of the resistant strain at all temperatures favorable for its development, and the number of deaths was strikingly fewer. Although at 27° and 30° there was a comparatively high percentage of disease in the Wisconsin Hollander plants, the amount of disease fell off rapidly at 24° and 33°, and at 21° none of the 10 plants was diseased. But at 24°, 27°, and 30° all of the susceptible plants were diseased, the number of diseased plants decreasing rapidly below 24°. At 18° there was no disease in either strain.

Something of this difference in susceptibility between the resistant and commercial strains is brought out in Figure 10. The three receptacles on the left in each case contain Commercial Hollander plants and the three on the right contain Wisconsin Hollander. Of the resistant plants shown in Figure 10, A (right), one was slightly diseased, whereas of the susceptible plants four were diseased and one was dead. At 30° (B) the disease was fatal to all plants of the susceptible strain and to two of the resistant strain, the other four

being diseased. At temperatures of 27°, 24°, and 21° the difference in susceptibility is so obvious as to need no discussion.

These results may be correlated with those obtained under field conditions. In general, the mean average soil temperature during the critical period of growth of the cabbage, June, July, and August, at Racine, Wis., lies between 18° and 24° C. If the mean average soil temperature remains about 20° to 21° there is little disease in the plants of resistant strains and from 40 to 80 per cent in those of the

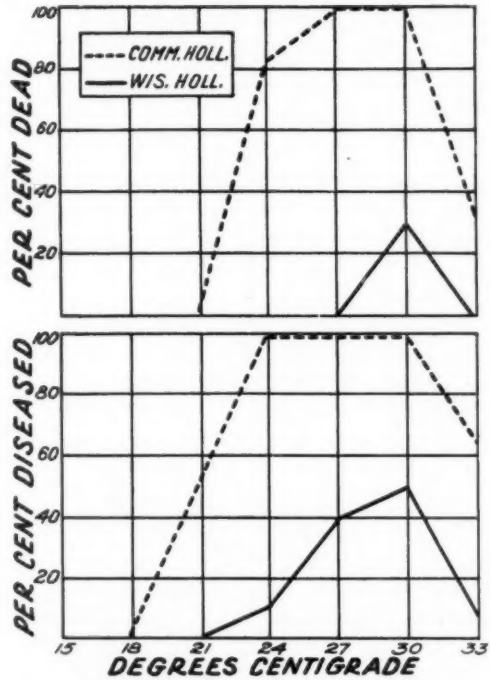

FIG. 9.—Comparison of yellows development in Wisconsin Hollander and Commercial Hollander plants grown at different temperatures for 30 days in naturally infested soil

susceptible strains. If the temperature goes up to about 24° the percentage of disease goes up also, these changes depending somewhat upon moisture as well as upon soil-temperature conditions.

RELATION OF SOIL MOISTURE TO THE DEVELOPMENT OF YELLOWS IN CABBAGE SEEDLINGS

Only one experiment was conducted to test the effect of soil moisture on the development of yellows in cabbage, and the results obtained were too variable to be of much value. But it may be mentioned that these results are in accord with those of Tisdale (*14*), who found that the disease is more severe in soils of comparatively low moisture content. Under conditions of proper soil moisture the cabbage plants seem to make better growth and are more resistant to the attack of the yellows fungus.

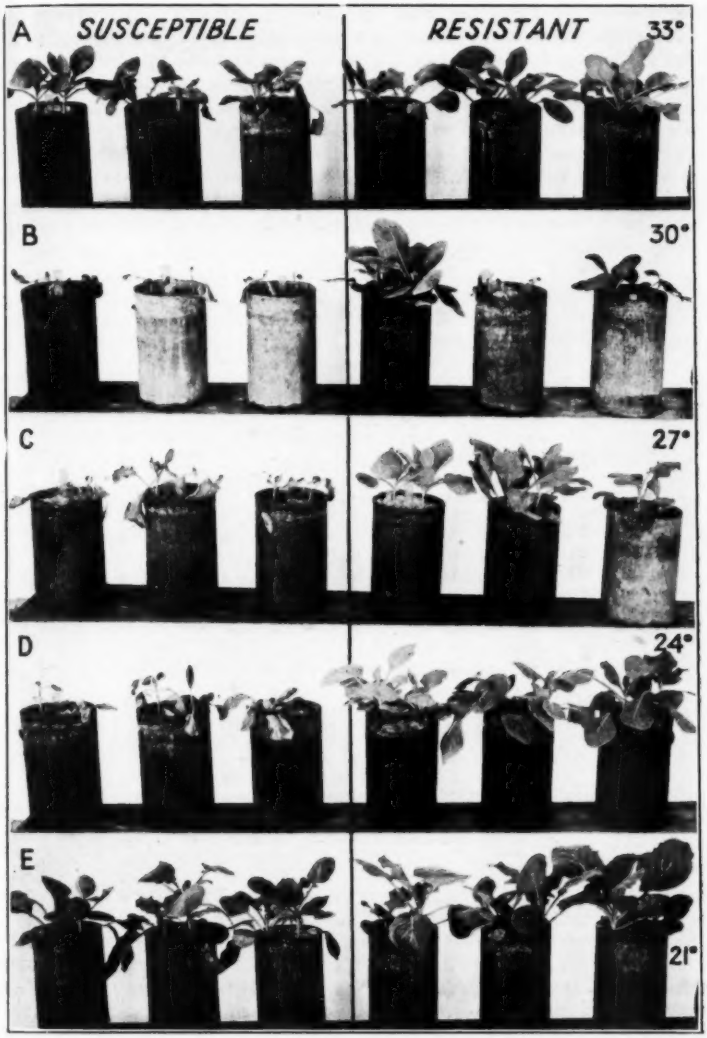

FIG. 10.—Wisconsin Hollander and Commercial Hollander plants grown in healthy soil for 62 days before being transplanted to diseased soil kept at 60 per cent of the moisture-holding capacity for 30 days at different temperatures. The three receptacles on the right contain in each case six plants of the resistant strain and the three on the left contain in each case six of the susceptible strain

A.—Soil temperature, 33° C. One of the resistant strain and four of the susceptible strain diseased; one of the latter strain dead

B.—Soil temperature 30° C. Four of the resistant variety diseased, two dead; all of the susceptible variety dead

C.—Soil temperature, 27° C. Four of the resistant strain slightly diseased, all the susceptible plants dead

D.—Soil temperature, 24° C. One of the resistant strain diseased, all of the susceptible strain dead

E.—Soil temperature, 21° C. All of the resistant strain healthy, and three of the susceptible variety diseased

It was noted by Jones and Gilman (*8*) and later by Tisdale (*14*) that severe attacks of yellows are associated with hot, dry weather. Gilman (*5*) showed that the incubation period of the disease ranged from 14 days, when the mean daily temperature 6 inches below the soil was 23° C., to 20 days when the mean daily temperature at the same depth was about 20°. In 1919 Tisdale transplanted Commercial Hollander seedlings to a soil where the daily temperature 4 inches below the surface was 25°, and 13 days later he found 30 per cent diseased. He concluded that the length of the incubation period might be as low as 12 days when the mean soil temperature was 20°.

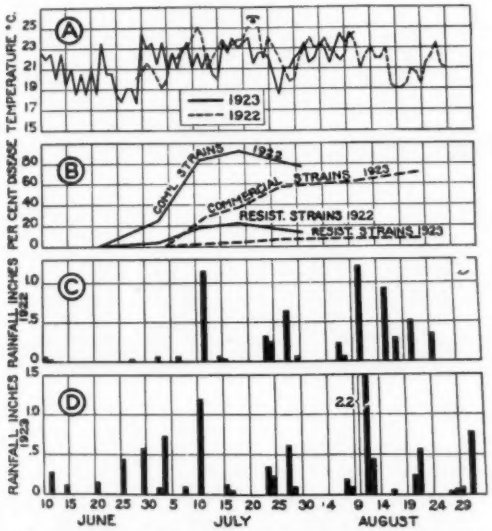

FIG. 11.—Summary of data from experimental plots at Racine, Wis., for June, July, and August, 1922 and 1923. A, mean average daily soil temperature at a depth of 4 inches; B, percentage of diseased plants in both resistant and susceptible strains; C and D, daily precipitation

He also found that lowering the moisture content of the soil increased to a considerable extent the quantity of disease.

Observations on the development of yellows in the experimental plots at Racine, Wis., in 1922 and 1923 made by the writer tend to corroborate those of Gilman and Tisdale. It was noted in 1922 that during a hot, dry period about the middle of July a comparatively high percentage of yellows developed in some of the most resistant strains, but that during the latter part of the month when the soil temperatures were considerably lower and rain fell abundantly many of the diseased plants completely recovered, and a little later when the counts of disease were made the actual percentage of yellows was lower than in the same plots two weeks earlier.

In correlating the temperature of the soil with the development of yellows in the experimental plots in 1922 and 1923, an attempt was made to ascertain whether the quantity of rainfall during July and August, the critical period for the development of the disease, might not have influenced the occurrence of Fusarium in the seedlings.

The results were not conclusive, but they rather indicate that the total rainfall during these months is a factor in the development of yellows. In 1922 the mean soil temperature for June, July, and August was about the same as for the same period in 1923, but the quantity of rainfall was much smaller in 1922, and the percentage of yellows in the experimental plots was much greater.

In 1922 some 32 strains were tested and in 1923 the number was increased somewhat, but only the more representative are included in the data given in Tables 8 and 9. The average percentage of disease in the resistant and suceptible strains is shown in Figure 11, B; the mean average soil temperatures at a depth of 4 inches from the surface for June, July, and August, 1922 and 1923, are given in Figure 11, A; and the total daily precipitation in inches, as taken from the United States Weather Bureau records (Racine office) for the same months in 1922 and 1923 (*15*), are shown in Figure 11, C and D.

Three features of Figure 11 are apparent at a glance: (1) The mean average soil temperature for the critical periods of 1922 and 1923 are very similar, the mean for the period being 21.1° C. in 1922 and 21.3° in 1923; (2) the amount of rainfall during the summer of 1922 was considerably less than that of 1923; and (3) the average percentage of yellows in both the resistant and susceptible strains was higher in 1922 than in 1923. Especially is this difference in disease evident in the resistant strains, the actual percentage being 13 in 1922 and 7.4 in 1923. This would seem to indicate that the decreased rainfall during the summer of 1922 might account in part at least for the increased percentage of disease over that of the following year.

TABLE 8.—*Summary of field observations on the experimental plots at Racine, Wis., 1922*

Strains	Total number of plants	July 3		July 11		July 19		July 31	
		Number diseased	Per cent diseased	Number diseased	Per cent diseased	Number diseased	Per cent diseased	Number diseased	Per cent diseased
Susceptible:									
All Seasons	254	31	12.0	153	60.0	188	74.0	180	70.0
Glory	246	47	19.0	181	73.0	217	88.0	210	85.0
All Head No. 1	247	66	26.0	196	79.0	197	79.0	186	75.0
All Head No. 2	89	38	42.0	78	87.0	82	92.0	74	83.0
Copenhagen No. 1	252	78	31.0	237	94.0	248	98.0	227	90.0
Copenhagen No. 2	119	66	55.0	114	96.0	118	99.0	117	98.0
Copenhagen No. 3	247	65	26.0	239	96.0	240	97.0	221	89.0
Copenhagen Iowa No. 5	121	10	8.0	52	43.0	55	45.0	60	50.0
Resistant:									
W. H. 9-21 (1-3)	555	3	.6	12	2.1	21	3.8	4	.7
W. H. 8-21 (A-B)	248	1	.4	10	4.0	13	5.2	8	3.2
XXV-20-A	483	1	.2	26	5.4	35	7.2	19	3.9
XXV-20-B	369	9	2.4	95	25.7	150	40.6	85	23.0
XXV-21-B	375	1	.3	40	10.6	68	18.1	30	8.0
XI-20 (1-2)	498	3	.6	76	15.2	124	24.9	56	11.2
XI-21 (2-5)	1,427	124	8.7	382	26.7	419	29.3	247	17.3
XXXV-20-4	349	44	12.6	130	37.2	145	41.5	107	30.6

TABLE 9.—*Summary of field observations on the experimental plots at Racine, Wis., 1923*

Strains	Total number of plants	July 11		July 18		July 26		Aug. 9		Aug 22	
		Number yellow	Per cent yellow	Number yellow	Per cent yellow	Number yellow	Per cent yellow	Number yellow	Per cent yellow	Number yellow	Per cent yellow
Susceptible:											
Glory	652	188	29.0	294	45.0	440	67.0	462	71.0	466	71.0
All Head	351	116	33.0	141	40.0	205	58.0	234	66.0	292	83.0
Mammoth Rock Red	366	63	17.0	104	28.0	113	30.0	140	38.0	190	52.0
Hollander	254	48	19.0	95	37.0	211	83.0	230	90.0	238	93.0
Brunswick	31	10	32.0			21	68.0	28	90.0	28	90.0
Large Blood Red	26	4	15.0	4	15.0	8	31.0	12	46.0	18	69.0
Resistant:											
W. H. 9-22-1	360	0	.0	3	.8	11	3.0	17	4.7	23	6.4
W. H. 8-22-A	170	0	.0	0	.0	1	.5	5	2.9	5	2.9
W. H. 8-22-D	253	1	.4	1	.4	3	1.2	17	6.7	28	11.0
XXV-22-A	545	2	.4	2	.4	2	.4	2	.4	2	.3
XXV-22 (1-6)	3,095	14	.5	18	.6	60	1.9	74	2.4	83	2.6
XXIV-22-2, 3, 4, 8	217	1	.4	1	.5	0	.0	0	.0	0	.0
XL-22-1	1,441	0	.0	0	.0	0	.0	0	.0	0	.0
XL-23-G. C	113							20	17.7	41	36.3
Red Nos. 1 and 2	809	13	1.6	20	2.5	58	7.2	60	7.4	63	7.8
XXXV-22-1	983	60	6.1	81	8.2	111	11.2	132	13.4	148	15.1
XXXV-22-2	1,179	64	5.4	81	6.9	158	13.4	165	14.0	178	15.1
XXXV-22-6	1,432	65	4.5	100	7.0	165	11.5	196	13.7	220	15.4
XXX-22-1	976	49	5.0	75	7.7	113	11.6	123	12.6	144	14.8
XXX-22-2	798	38	4.7	50	6.3	79	9.9	100	12.5	111	13.9
XXX-22-4	710	30	4.2	41	5.7	52	7.3	61	8.6	67	9.4

SUMMARY

In the experiments herein described the writer has studied the effect of environmental factors upon the expression of the resistant quality in cabbage exposed to attacks by the fungus *Fusarium conglutinans*.

It was found that young seedlings of Wisconsin All Seasons and Commercial All Seasons strains were almost equally susceptible to yellows up to the age of 20 days.

A high percentage of plants of the most resistant varieties became diseased when placed under conditions of low soil moisture and relatively high soil temperature, 27° to 33° C., but from 21° to 24°—the temperatures nearest those encountered in the field in the Racine, Wis., cabbage-growing section—the "resistant" varieties contracted the disease only slightly. No evidence of yellows development was noted at a soil temperature of 15° and only a comparatively small percentage developed at 18°.

Cabbage plants of both "resistant" and "susceptible" strains grown in healthy soil at 12° to 16° C. before transplantation to diseased soil at a higher temperature (27°) proved to be more susceptible to yellows than plants grown at 25° to 28°.

Air temperature affects yellows development independently of soil temperature. Wisconsin Hollander and Commercial Hollander plants were grown in naturally infested soil at soil temperatures of 15°, 21°, and 27° C. in each of three greenhouse rooms kept at 14.5°, 19° and 25°, respectively, for 30 days. At a soil temperature of 27° the disease developed most rapidly in the 25° chamber and slightly more slowly in the 19° and 14.5° chambers, but the final percentage

of disease was about equal in the three. At a soil temperature of 21° the disease developed most rapidly in the 25° room and less rapidly in the 19° room, and the disease was very slight in the 14.5° room.

Commercial Hollander plants were grown in diseased soil at a soil temperature of 21° C. and at air temperatures of 14° and 28°. Yellows developed much more rapidly, a larger percentage of plants were diseased, and a much greater number of plants were killed by the disease at an air temperature of 28° than 14°.

A study of field conditions for two years and a limited amount of greenhouse experimentation show that the quantity of rainfall apparently affects the amount of disease independently of soil temperature relations. In the summer of 1922 the soil temperatures at Racine, Wis., averaged somewhat lower than in 1923, but the total percipitation was considerably less in 1922 and the average percentage of disease was considerably higher.

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